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Clinical Pharmacology and Pharmacotherapy

INFLUENCE OF GASTRIC BYPASS ON NUTRIENT INTAKE AND ORAL DRUG DISPOSITION

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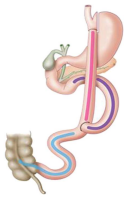

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LIST OF ABBREVIATIONS

AACE	American Association of Clinical Endocrinologists
ASMBS	American Society for Metabolic & Bariatric Surgery
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
BCS	Biopharmaceutical Classification System
BIA	Bioelectrical Impedance Analysis
BID	Bis In Die
BMI	Body Mass Index
BMR	Basal Metabolic Rate
CI	Confidence Interval
C_{max}	Maximum (or peak) serum concentration
CPAP	Continuous Positive Airway Pressure
CR	Controlled release
CRP	C-reactive Protein
CVD	Cardiovascular Diseases
CYP	Cytochrome P450
DBP	Diastolic Blood Pressure
DMSO	Dimethyl Sulfoxide
DRV	Darunavir/Ritonavir
DXA	Dual-energy X-ray Absorptiometry
EAR	Estimated Average Requirements
EASO	European Association for the Study of Obesity
EI	Energy Intake
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicines Agency
EN%	Energy Percentage
ENG	Etonorgestrel
EudraCT	European Union Drug Regulating Authorities Clinical Trials
EURRECA	European Micronutrient Recommendations Aligned
EWL	Excess of Weight Loss
FA	Fatty Acid
FCDB	Food Composition Database
FDA	Food and Drug Administration
FMI	Fat Mass Index
FFMI	Fat Free Mass Index

FTC	Emtricitabine
GI	Gastrointestinal
GLP-1	Glucagon-Like Peptide-1
GP	General Practitioner
H	Hospital
HCP	Health Care Professional
HCQ	Hydroxychloroquine
HIV	Human Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
HPMCAS	Hypromellose Acetate Succinate
HRM	High-Resolution Manometry
IFSO-EC	European Chapter if the International Federation of Surgery
INR	International Normalized Ratio
IOM	Institute of Medicine
IR	Immediate Release
IU	International Unit
IVIVC	*in vitro-in vivo* correlation
k_{in}	First-order transfer rate constant from central to peripheral compartment
k_{out}	First-order transfer rate constant from peripheral to central compartment
LT4	Levothyroxin
M	Month
MSM	Multiple Source Method
MTX	Methotrexate
NIHDI	National Institute for Health and Disability Insurance
NS	Not Significant
NSAID	Non-Steroidal Anti-Inflammatory Drug
OAD	Oral Antidiabetic
OMTF	Obesity Management Task Force
OR	Odds ratio
OSA	Obstructive Sleep Apnea
PAL	Physical Activity Level
PBPK	Physiologically-based pharmacokinetic
PK	Pharmacokinetic
PPI	Proton Pump Inhibitor
RDA	Recommended Dietary Allowances
RYGB	Roux-en-Y Gastric Bypass
SBP	Systolic Blood Pressure
SEM	Standard Error of the Mean

SD	Standard Deviation
SNRI	Serotonin-Norepinephrine Reuptake Inhibitor
SOS	Swedish Obese Subjects
SRI	Serotonin Reuptake Inhibitor
SRL	Sirolimus
SSRI	Selective Serotonin Reuptake Inhibitor
T2DM	Type 2 Diabetes
TDF	Tenofovir Disoproxil Fumarate
T_{max}	Time at which C_{max} is reached
TOS	The Obesity Society
TSAT	Transferrin Saturation
USA	United States of America
V_D	Volume of Distribution
V_{sac}	Volume of single adjusting compartment
V_{ss}	Volume of distribution at steady state
WHO	World Health Organization
Y	Year

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PART I: GENERAL INTRODUCTION AND OBJECTIVES

CHAPTER I: GENERAL INTRODUCTION

1 GENERAL INTRODUCTION

1.1 Obesity and bariatric surgery

The prevalence of overweight and obesity has increased to epidemic proportions and has become a global health problem, affecting millions of people. The World Health Organization (WHO) defines overweight and obesity as an abnormal or excessive fat accumulation, which presents a risk for health [1]. The standard parameter to determine obesity, is Body Mass Index (BMI), corresponding to weight (kg) divided by the square of the height (m^2). Based on BMI, overweight and obesity can be classified in different categories, as shown in Table 1.

Table 1: Classification of overweight and obesity, based on BMI [2]

Classification	**BMI (kg/m^2)**
Healthy weight	18.5–24.9
Overweight	25–29.9
Obesity I	30–34.9
Obesity II	35–39.9
Obesity III	40 or more

Health consequences of obesity are more serious when the degree of overweight increases. However, health consequences are also dependent on the distribution of body fat, as people with an excess of visceral fat have a higher risk for the development of health problems. Waist circumference is the best anthropometric indicator for visceral fat [3].

Health consequences of obesity include type 2 diabetes mellitus (T2DM), cardiovascular diseases, dyslipidemia, and obstructive sleep apnea [4]. These obesity-related diseases result in an increased consumption of drugs in this population [5] and in an increase in mortality. Therefore, both prevention of obesity and effective treatment are essential [4].

To treat obesity, lifestyle interventions including smaller caloric intake and more physical activity, remain essential. However, results on the long-term of lifestyle interventions as such, are often

disappointing [6]. Pharmacotherapy has also shown limited efficacy on both the extent of weight loss and the effect on the long term [7].

Nowadays, bariatric surgery, in combination with lifestyle modifications, is the most effective treatment for morbid obesity to obtain major and sustainable weight reduction [4]. Furthermore, it results in an improvement of several obesity-related diseases such as T2DM, hypertension and sleep apnea and in a significant reduction in mortality [8-10]. However, bariatric surgery can also be associated with complications on the short and long-term, including gastrointestinal and nutritional complications [11]. Therefore, a lifelong follow-up is essential.

In Belgium, to receive reimbursement from the National Institute for Health and Disability Insurance (NIHDI) for the costs associated with bariatric surgery, patients need to meet the following conditions:

- older than 18 years
- BMI≥40 kg/m², or BMI≥35 kg/m² in patients with comorbidities (T2DM, sleep apnea or therapeutic resistant hypertension) and in patients who underwent a previous unsuccessful bariatric procedure
- followed a documented diet during one year that was unsuccessful
- multidisciplinary approval from a surgeon, internist and psychologist/psychiatrist

Different bariatric procedures are available, which can be classified in three groups: (1) restrictive procedures, resulting in a limitation of food intake; (2) malabsorption procedures, resulting in a reduced absorption from the intestine; and (3) procedures based on the combination of both restriction and malabsorption.

Nowadays, a Roux-en-Y Gastric Bypass (RYGB) is the most commonly performed bariatric procedure, which is based on a combination of restriction and malabsorption (see Figure 1). In a RYGB, a small gastric pouch with a volume of 15 to 30 mL and a lower gastric remnant is formed by staples. The jejunum is divided 30 to 75 cm distal to the ligament of Treitz and the distal part is connected with

the newly formed gastric pouch, which forms the 'Roux-limb'. The gastric remnant and bypassed biliary limb is reconnected to the intestine, 75 to 150 cm distal to the anastomosis between the gastric pouch and the distal part of the jejunum [12].

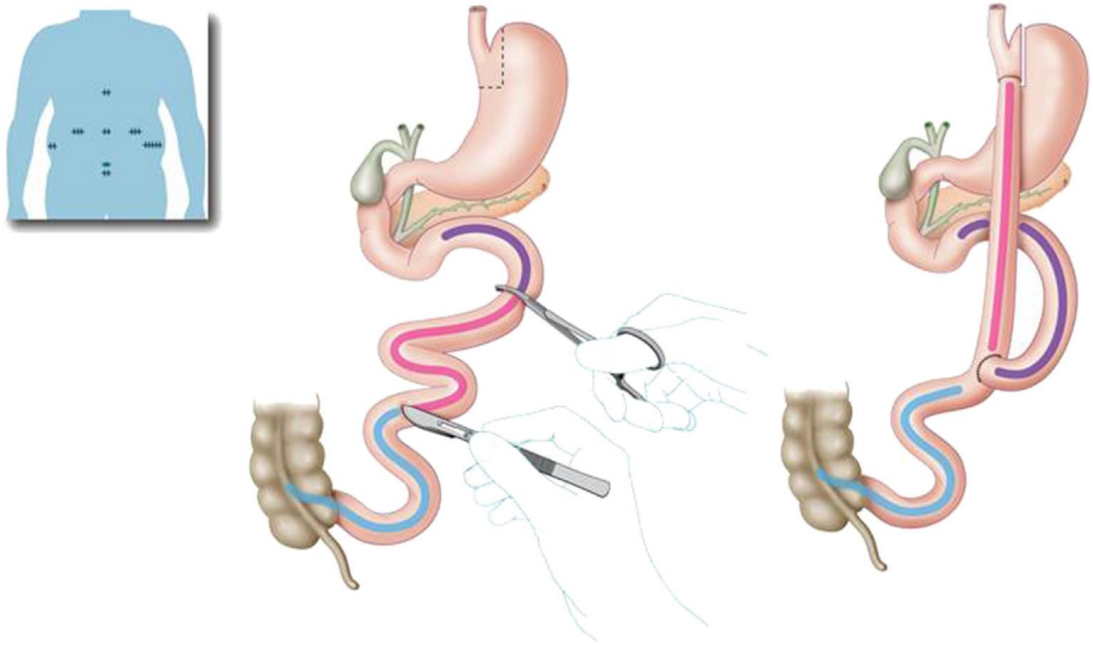

Figure 1: Roux-en-Y Gastric bypass [13]
[Figure adapted from Lannoo et al., with permission from Best Pract Res Clin Gastroenterol]

Patients with a RYGB will experience an early satiety due to the small gastric pouch, resulting in a decreased food intake. Furthermore, there is less absorption of food as the proximal part of the small intestine is bypassed resulting in a reduced absorption surface. Both factors contribute to weight loss after RYGB. However, these factors will also influence the intake and absorption of macro- and micronutrients and drugs.

1.2 Influence of RYGB on the absorption of macro- and micronutrients

Bariatric surgery leads to anatomical changes of the gastrointestinal tract, which can influence the absorption of nutrients. The most important factors that will influence the absorption of nutrients after RYGB are (1) the bypass of the proximal part of the small intestine, which is the major absorption site for numerous micronutrients as shown in Figure 2; (2) the reduced gastric acid secretion; and (3) the reduced secretion of intrinsic factor [14].

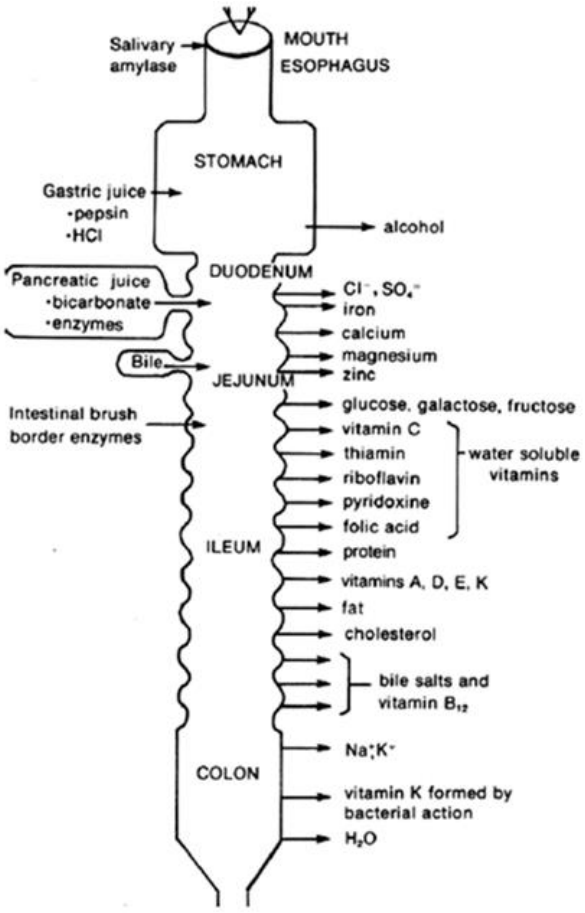

Figure 2: Absorption sites in the gastrointestinal tract of vitamins, minerals and nutrients [15]
[Figure adapted from Bloomberg et al., with permission from Obes Surg]

1.2.1 Macronutrients

PROTEINS

Proteins are digested by the enzyme pepsin. After RYGB, levels of pepsinogen, the inactive precursor of pepsin, are significantly reduced compared to baseline values [16]. The conversion of pepsinogen to the active pepsin is facilitated by gastric acid, the secretion of which is also reduced after RYGB. Furthermore, the inlet of secreted pepsin and other digestive enzymes is delayed after RYGB. These factors may result in a reduced digestion of proteins after surgery.

Odstrcil et al. [17] have shown that in patients who did not take protein supplements (n=7), protein absorption was decreased at 5 and 14 months post-RYGB compared to baseline values. Nevertheless, two patients who were taking protein supplements had higher protein absorption after RYGB than before surgery.

CARBOHYDRATES

To examine carbohydrate absorption, Wang et al. [18] have performed a D-xylose test in seven morbidly obese patients before and one year post-RYGB. No significant differences between pre- and postoperative D-xylose serum concentrations were observed, so no carbohydrate malabsorption occurred one year post-RYGB. Furthermore, Odstrcil et al. [17] have shown that there were only small changes in the carbohydrate absorption coefficient after RYGB.

LIPIDS

After RYGB, the inlet of lipolytic enzymes and bile acids is delayed, so the digestion of lipids and the formation of micelles is delayed, which can lead to fat malabsorption [19].

Kumar et al. [20] have demonstrated that the fecal fat excretion (n=9) was increased in patients 6 and 12 months after RYGB compared with measurements before surgery, even though the fat intake was decreased. This has been confirmed by Carswell et al. (n=7 post-RYGB patients; n=7 obese controls) [21] who performed a 3-day collection of faeces and by Odstrcil et al. (n=9) [17] who showed that the fat absorption coefficient decreased from 92.1% before surgery to 71.9% and 68.1% 5 and 14 months post-RYGB, respectively. Fat absorption decreased with a higher magnitude than protein absorption.

1.2.2 Micronutrients

Patients with a RYGB have an increased risk for the development of micronutrient deficiencies. Ledoux et al. [22] have analyzed the nutritional status of patients before, and 1 and 3 years post-RYGB. The percentages of patients with a specific nutritional deficiency at each time point, are shown in Table 2.

Table 2: Percentage of patients with specific deficiencies before and after RYGB [22]
[Table adapted from Ledoux et al., with permission from Ann Surg]

	Before Surgery, N = 144	At 1 yr, N = 144	≥3 yr, N = 115
Percentage of subjects with parameters < normal value			
Albumin <34 g/L	8	6	7
Serum calcium <2.12 mmol/L	5	6	1
Hemoglobin <11.5 g/dL	6	12	13*
Tranferrin saturation <20%	42	34†	50‡
Vitamin B_1 <126 nmol/L	22	16*	27‡
Vitamin B_6 <20 nmol/L	24	17†	17†
Vitamin B_9 <3 μg/L	2	4	1
Vitamin B_{12} <190 ng/L	5	16*	11*
Vitamin C < 5 mg/L	33	14§	11§
Vitamin A < 1.5 μmol/L	10	19	14
Vitamin E < 21 μmol/L	15	23	26*
Vitamin 25OHD <30 μg/L	92	86†	71†‡
Total no. of deficiencies (n)	3.2 ± 2.3	3.4 ± 2.0	3.5 ± 23

Values are percentage of subjects with deficiencies defined as values below the low normal value of the laboratory.
*$P < 0.05$ versus baseline.
†$P < 0.01$ versus baseline.
‡$P < 0.05$ versus 1 yr.
§$P < 0.001$ versus baseline.

IRON

The prevalence of iron deficiency after RYGB ranges from 20% to 49% [23]. There are three factors that contribute to the development of an iron deficiency post-RYGB: (1) reduction of gastric acid secretion, which is required for the absorption of iron as it transforms the ferric form (Fe^{3+}) to the ferrous form (Fe^{2+}), which is the absorbable form; (2) diminished intestinal absorption surface, especially the bypass of the duodenum, which is the main absorption site of iron; and (3) low tolerance to red meat, which is an important source of iron [11]. Ruz et al. (n=67♀) have shown that the iron absorption is decreased after RYGB [24]. The absorption from a standard diet and from a standard dose of ferrous ascorbate was significantly reduced 6 months post-RYGB to 32.7% and 40.3% of baseline values respectively. They observed no further significant changes of the absorption 12 and 18 months post-RYGB. Furthermore, in another study performed by Ruz et al. (n=32♀), the absorption of both haem- and non-haem iron was reduced 1 year after RYGB [25]. The extent of the reduced absorption was greater for haem iron than for non-haem iron [25].

Rosa et al. [26] performed iron tolerance tests (n=9♀) before and 3 months post-RYGB and demonstrated a delayed iron absorption post-RYGB. The first hour after administration, the plasma concentration of iron was significantly lower after surgery. However, the total iron concentration until 4 hours after administration was not significantly lower after RYGB, even though 6 of the 9 patients presented a mean decrease in AUC of 51%.

ZINC

A zinc tolerance test (n=9♀) was performed before and 3 months after surgery and showed that the plasma zinc concentrations were significantly lower after RYGB [26]. In another study, the percentage of zinc absorption also decreased significantly after RYGB (n=67♀). Before surgery the absorption percentage was 32.3%; 6 and 18 months post-RYGB it was decreased to 13.6% and 21%, respectively [27]. As shown in Figure 3, zinc is mainly absorbed in the duodenum and proximal parts of the jejunum. Bypassing these proximal parts of the small intestine along with the reduced intestinal surface area can contribute to the reduced absorption capacity for zinc.

CALCIUM

Riedt et al. (n=21♀) have demonstrated that 6 months after RYGB, the true fractional absorption of calcium from milk was significantly reduced. They observed a 34% reduction after surgery, but the values remained within normal range for most women as the true fractional calcium absorption before surgery was relatively high [28]. Moreover, it seems that calcium citrate is better absorbed after RYGB than calcium carbonate as the mean serum concentration and peak plasma concentration of calcium were significantly higher after oral administration of calcium citrate compared to calcium carbonate, [29]. This can be explained by the fact that calcium carbonate is more dependent on gastric acid secretion for its absorption than calcium citrate. When starting calcium supplements in RYGB-patients, preference should be given to supplements containing calcium citrate.

VITAMIN D

Regarding the absorption of vitamin D, one study showed that after oral administration of 50 000 IU solubilized cholecalciferol (n=14♀), the peak plasma concentration (C_{max}) of cholecalciferol was decreased with 27% in patients 4 weeks after RYGB compared to preoperative values. However, there were no significant differences in the area under the curve ($AUC_{0\text{-}72h}$), which represents the total drug exposure over time [30]. This may be an underestimation of the decreased absorption of vitamin D as weight loss after RYGB results in a decreased distribution volume, which will cause an increase in C_{max} and AUC.

VITAMIN B_{12} AND FOLIC ACID

The anatomical changes associated with RYGB, can cause the development of vitamin B_{12} deficiency. Smith et al. (n=7♀, 3♂) have shown that food-bound vitamin B_{12} is poorly digested and subsequently poorly absorbed after RYGB [31]. However, the absorption of free vitamin B_{12} was normal after surgery. The reduced absorption of food-bound vitamin B_{12} can be explained by the reduced secretion of gastric acid, which is essential for the release of vitamin B_{12} from proteins. Furthermore, the secretion of intrinsic factor is decreased, which is also necessary for the absorption of vitamin B_{12}. Smith et al. [31] have shown a correlation between the absorption of food-bound vitamin B_{12} and the basal acid secretion from the gastric pouch. Behrns et al. (n=6♀, 2♂) [32] have performed a comparable study and also showed that the gastric acid secretion was significantly decreased post-RYGB resulting in a significant decrease of the absorption of food-bound vitamin B_{12}; the absorption of vitamin B_{12}, administered as crystalline vitamin B_{12}, was also decreased, but no significant differences were described.

The absorption of folic acid occurs mainly at the level of the proximal part of the small intestine. However, after surgery folic acid can be absorbed through all the small intestine because of adaptation [33]. If folic acid deficiency after RYGB is developed, it is caused by a reduced intake of folic acid.

1.3 Influence of RYGB on the intake of macro- and micronutrients

Obese patients consume high fat diets, which have often a low content of micronutrients. It has been shown that the prevalence of nutritional deficiencies, including iron, vitamin B_1 and vitamin D deficiency, is already higher in obese individuals than in normal weight individuals [34;35]. This is called 'double burden' of malnutrition. So, before bariatric surgery, patients are already at risk for nutritional deficiencies and therefore, there is strong evidence for an extensive preoperative nutritional screening [36]. This allows treatment of deficiencies before surgery. The latter is important as a recent study has shown that preoperative micronutrient deficiencies persist after surgery and, moreover, that many patients develop new deficiencies, including iron and vitamin B_{12} deficiencies [37].

Patients with a RYGB consume less food after the operation, which is associated with a reduced intake of macro- and micronutrients. Furthermore, food preferences change after RYGB and some patients avoid certain foods because of the development of food intolerances such as for red meat, which is a major source of iron. This may further contribute to the reduced intake of some micronutrients postoperatively [38;39]. In what follows, we give an overview of the data from the literature about the influence of gastric bypass on the intake of macro- and micronutrients.

1.3.1 Macronutrients

Previous studies have shown that the energy intake is enormously reduced immediately after RYGB, which is due to a reduction in carbohydrate, protein and fat intake [17;28;40-42]. Caloric intake increases again with time after surgery, but remains below baseline values until 5 years post-RYGB [42]. The same effects have been found for the intake of macronutrients, suggesting an increase in gastric capacity and consequently tolerance of larger food amounts during the years following RYGB.

PROTEINS

A study, performed in patients at least 18 months post-RYGB (n=68), showed that about 22% from the energy intake was from proteins [43]. Moizé et al. [44] have shown that the percentage of patients

with a protein intake below 60 g was 52% and 60%, 4 and 12 months post-RYGB, respectively. They indicated that the consumption of insufficient amounts of proteins was due to protein intolerance at one year post-RYGB [45]. In the study of Mercachita et al. [41] 62.2% (n=28) and 29.4% (n=5) of the patients had a protein intake below the minimal recommended intake (0.8 g/kg) 1 and 2 years post-RYGB, respectively. Protein intake is very important as there is a significant negative correlation between daily protein intake and the percentage of lean tissue mass loss 4 and 12 months post-RYGB [44].

CARBOHYDRATES

By performing a 24h recall study in patients who underwent RYGB more than 18 months ago (n=68), Wardé-Kamar et al. [43] have shown that about 44% of the energy intake was derived from carbohydrates. Comparable results were shown by Torres Rossi et al. [46]. The carbohydrate intake in the latter study varied between 41.5 and 53.0% of the caloric intake in patients post-RYGB.

More specifically, Miller et al. [40] have shown that the dietary fiber and sugar intake was lower until one year post-RYGB compared to preoperatively. A high percentage of the participants (94–100%) had an inadequate fiber intake before and at 1, 3, 6 and 12 months post-RYGB. This low dietary fiber intake after RYGB was confirmed by Novais et al. [47].

LIPIDS

In patients at least 18 months post-RYGB (n=68), about 33% of the energy intake derived from fat [43]. Kumar et al. have collected food frequency questionnaires (n=9♀, 2♂) and showed that the dietary fat intake decreased significantly post-RYGB [20]. Before surgery, the mean fat intake was 79.5 g and decreased to 39.3 and 50.9 g 6 and 12 months post-RYGB, respectively.

1.3.2 Micronutrients

Table 3 gives an overview of all studies that have investigated the influence of RYGB on the intake of micronutrients. The conclusions of these studies are summarized below, with a specific focus on iron, calcium, vitamin B_1, B_{12} and D deficiencies that are the most common deficiencies after RYGB.

IRON

In a study performed by Colossi et al. [48], 24h dietary records of 210 patients at different time points after RYGB (1, 3, 6, 9, 12, 18 and 24 months) have been collected. The intake of iron increased during the study period, but the minimal requirements for iron were not obtained.

However, Wardé-Kamar et al. [43] have shown that the dietary iron intake met the Recommended Daily Allowances (RDA) or was even higher in patients at least 18 months post-RYGB.

CALCIUM

Recently, Miller et al. [40] have conducted a study in which 17 patients completed a food record during 4 days before and 3 weeks, 3 months, 6 months and 12 months after RYGB. For more than 50% of the participants, the calcium intake one year after RYGB was lower than the Estimated Average Requirements (EAR). The lower dietary intake of calcium postoperatively has been confirmed by Riedt et al. [28]. Furthermore, in other studies it has been shown that the minimal requirements for calcium were not obtained post-RYGB [48;49]. However, in the study of Mercachita et al. [41] there were no significant differences in the intake of calcium before and after RYGB, but a lot of patients did not reach RDA before, one year and two years post-RYGB: 57% (n=34), 71% (n=32) and 65% (n=11), respectively. Similar results were obtained in patients more than 18 months post-RYGB, their dietary calcium intake was only 68 ± 47% of the RDA [43].

VITAMIN B_1 AND B_{12}

A cross-sectional study was performed among 44 women after RYGB (mean time after surgery was 3.4 years) and 38 women in a control group, who were matched by age and economic condition to collect 4-day records [46]. A lower intake of vitamin B_1 and B_{12} in the women with RYGB compared to those without RYGB was observed. Colossi et al. [48] have shown that the minimal requirements for vitamin B_1 intake were not obtained after RYGB. However, Wardé-Kamar et al. [43] have shown that the dietary vitamin B_{12} intake met the RDA or was even higher in patients at least 18 months after surgery.

VITAMIN D

Only one study specifically investigating the intake of vitamin D was found in literature. In this study, the intake of vitamin D was lower than the EAR for more than 50% of the participants (n=17) one year post-RYGB [40].

OTHER MICRONUTRIENTS

Mercachita et al. [41] have shown a significant reduction of folic acid intake one year after RYGB compared to the preoperative intake. After one year, the intake increased again until two years postoperatively, but did not reach significance. This has been confirmed by a study of Wardé-Kamar et al. [43], in which the dietary folic acid intake was only 61 ± 37% of the RDA in patients who underwent a RYGB more than 18 months before. Miller et al. [40] have compared the folic acid intake with the EAR and one year post-RYGB, more than 50% (n=17) did not reach the EAR.

Miller et al. [40] have also investigated the influence of RYGB on vitamin C intake. The intake of vitamin C was reduced 3 weeks post-RYGB, but the intake gradually increased to one year post-RYGB. One year post-RYGB, the intake was lower than the EAR for more than 50% of the participants (n=17) [40]. The lower dietary intake of vitamin C postoperatively has been confirmed by Netto et al. [50]. Furthermore, 4-day food records in 44 women three years post-RYGB showed that the intake of vitamin C did not meet the EAR [46].

Compared to the EAR, the intake of vitamin E was lower in more than 50% of the participants (n=17) one year post-RYGB [40;46]. Regarding vitamin A, Collosi et al. have shown that the vitamin A intake did not meet the minimal requirements, based on 24h food records (n=210 patients) [48]. Furthermore, the intake of vitamin A did not meet the EAR in women post-RYGB (n=44), neither in a control group with healthy women (n=38) [46].

By investigating the intake of magnesium and potassium before and after RYGB by Miller et al. [40], it has been shown that the intake of magnesium and potassium did not reach the EAR in more than

50% of the patients post-RYGB [40]. Cominetti et al. [51] have observed that there was a reduced intake of zinc two months after RYGB compared with the intake before surgery.

Table 3: Overview of performed studies regarding influence of RYGB on micronutrient intake

Reference	Micronutrients studied	Methodology	Number of subjects	Time points studied	Effect on intake
Cominetti et al. [51]	Zinc	3-day food record	24 patients (20♀, 4♂)	Before RYGB 2 months post-RYGB	Statistically significant reduction of zinc intake post-RYGB in comparison with the preoperative intake. Before RYGB: 56% of patients zinc intake > EAR 2 months post-RYGB: 31% of patients zinc intake > EAR
Colossi et al. [48]	Vitamin A, B_1, B_2, B_3, B_5, B_6, B_9 B_{12}, C, calcium and iron	24h food recall	210 patients (147♀, 63♂)	1, 3, 6, 9, 12, 18 and 24 months post-RYGB	The intake of micronutrients increased with time after surgery. Vitamin A, B_1, B_3, B_5, B_6, B_9, C and iron: the minimal requirements were not attained at the different time points post-RYGB
de Torres Rossi et al. [46]	Vitamin A, B_1, B_{12}, C, E, calcium, iron and zinc	4-day food record	44 patients post-RYGB (♀) 38 age and economic condition matched controls (♀)	At least 1 year post-RYGB	Vitamin B_1 and B_{12}, iron and zinc: intake was significant lower post-RYGB Vitamin A and E: intake was below EAR in both groups Vitamin C: intake was below EAR in RYGB-patients and between EAR and RDA for the control group Vitamin B_{12}: post-RYGB, the intake was between EAR and RDA post-RYGB and above RDA in the control group
Duran de Campos et al. [49]	Calcium	Food frequency questionnaire and 3-day dietary recall	30 patients (♀)	At least 8 years post-RYGB	The intake of calcium was below RDA (for both methods).
Mercachita et al. [41]	Vitamin B_{12}, folic acid, iron and calcium	24h food recall	60 patients (39♀, 21♂)	Before RYGB 1 year post-RYGB 2 years post-RYGB	Vitamin B_{12}, folic acid and iron intake: significant reduction 1 year post-RYGB in comparison with intake before RYGB; intake increased again 2 years post-RYGB (NS) Calcium intake: comparable trend, but no significant differences The percentage of patients not meeting RDA increased after RYGB; 1 and 2 years after RYGB, more than 65% of the patients had an intake below DRI for vitamin B_{12}, folic acid, iron and calcium.

Miller et al. [40]	Vitamin A, B_1, B_2, B_3, B_6, B_{12}, C, D, E, K, folic acid, calcium, copper; iron, magnesium and zinc	4-day food record	17 patients (16♀, 1♂)	Before RYGB 3 weeks post-RYGB 3, 6 and 12 months post-RYGB	Intake of micronutrients was the highest before RYGB, the lowest 3 weeks post-RYGB and increased gradually from 3 weeks to 12 months post-RYGB, some returning to the values before RYGB. The percentage of patients not meeting EAR increased after RYGB. One year post-RYGB, more than 50% of the patients had an intake below EAR for vitamin C, D, E, folic acid, calcium, magnesium and potassium.
Moizé et al. [42]	Calcium, phosphorus, magnesium and iron	3-day food record	294 patients (226♀, 28♂)	Before RYGB 3, 6, 12, 18, 24, 30, 36, 48 and 60 months post-RYGB	The mean dietary intake of the studied micronutrients was below RDA.
Netto et al. [50]	Vitamin C	Food frequency questionnaire	26 patients post-RYGB 26 sex- and age- matched controls (22♀, 4♂)	Before RYGB 1 year post-RYGB 2 years post-RYGB	Intake of vitamin C was significantly lower 1 and 2 years post-RYGB compared to baseline values.
Riedt et al. [28]	Calcium	3-day food record	25 patients (21♀, 4♂)	Before RYGB 6 months post-RYGB	The dietary calcium intake was significantly reduced 6 months post-RYGB compared to the intake before RYGB.
Wardé-Kamar et al. [43]	Vitamin B_{12}, folic acid, calcium and iron,	24h food recall	69 patients (61♀, 5♂)	At least 18 months post-RYGB	Vitamin B_{12} and iron: dietary intake was at or above RDA Folic acid and calcium: dietary intake was 61±37% and 68±47% of the RDA, respectively.

EAR = Estimated Average Requirements
RDA = Recommended Dietary Allowances

1.4 Medication use before and after Roux-en-Y gastric bypass

Obese patients are prone to an increased consumption of drugs compared to lean individuals as obesity is associated with a lot of comorbidities, as mentioned before [5]. After RYGB, a lot of obesity-related diseases improve, resulting in a decreased consumption of related treatments [9;52]. A study from Segal et al. [53] has shown that the consumption of drugs used for T2D, dyslipidemia and hypertension reduced significantly as soon as 1 year post-RYGB by 76, 59 and 51%, respectively. This reduction remained significant until 3 years post-RYGB [52]. In the Swedish Obese Subjects (SOS) study, patients with RYGB were compared with a matched control group regarding costs associated with drug use for obesity-related diseases. After 7 years, drug costs were significantly lower for patients with RYGB and this reduction continued to be significant during 20 years of follow-up [54].

In contrast to the improvement of obesity related diseases, RYGB can also induce potential risks including nutritional deficiencies (See 1.3) and surgical complications on the short term, such as venous thrombosis, anastomosis ulceration and gallstones [55]. These risks can be prevented by specific treatments, which may consequently increase drug consumption [55;56].

Furthermore, RYGB changes the anatomical structure of the gastrointestinal tract and subsequently its physiology. Therefore, the choice of medication in this population group needs to be considered as well [57]. For example, patients with a RYGB have an increased risk for gastrointestinal ulceration because of the reduced stomach size. Therefore, drugs that are associated with an increased risk of ulcers such as NSAIDs and bisphosphonates need to be avoided in this population group [57].

These anatomical and physiological changes may also alter drug disposition after RYGB, which can lead to a reduced efficacy of treatment or to toxic drug levels. If a decreased efficacy of a drug is observed after RYGB, formulation (i.e. immediate release formulation instead of an extended release formulation, or a liquid formulation instead of a tablet formulation) or administration route changes (i.e. transdermal, subcutaneous) might be necessary. In the next section, we will focus on the bioavailability of drugs after RYGB.

1.5 Influence of RYGB on the bioavailability of orally administered drugs

As explained before, obesity is often associated with multiple chronic comorbidities resulting in a high consumption of medication. The bioavailability of these drugs may be altered after bariatric surgery. This might also be true for drugs that are given in an acute situation such as antibiotics.

The solubility of drugs and the permeability of the intestine determine drug bioavailability. Drug bioavailability is further influenced by physiological factors, which may be affected by RYGB as it alters the anatomical structure and physiology of the gastrointestinal tract including changes in gastric pH, gastric emptying, inlet of bile acids and transit time. The oral drug bioavailability will be determined by the interplay between these physiological and physicochemical factors.

The section below gives a detailed overview of what is known about physiological changes after RYGB influencing oral drug bioavailability. Furthermore, data on the impact of RYGB on oral drug bioavailability are summarized. A good insight in these changes is important as they can result in potentially dangerous over- or underdosing.

1.5.1 Changes of physiological factors influencing drug bioavailability after RYGB

RYGB results in a decrease of the volume of the stomach, which has an impact on gastric mixing and production of gastric acid. Furthermore, a new connection is formed between the stomach and the intestine, which has an effect on gastric emptying. Additionally, a lot of anatomical and physiological factors are changed at the level of the small intestine as the duodenum and proximal part of the jejunum are bypassed, and reconnected more distally to the small intestine which has an impact on the surface area for absorption, on the intestinal transit time, bile acid solubilization and enterohepatic recirculation, on receptors, transporters and metabolism in the small intestine, as shown in Figure 3. All these changes are discussed below.

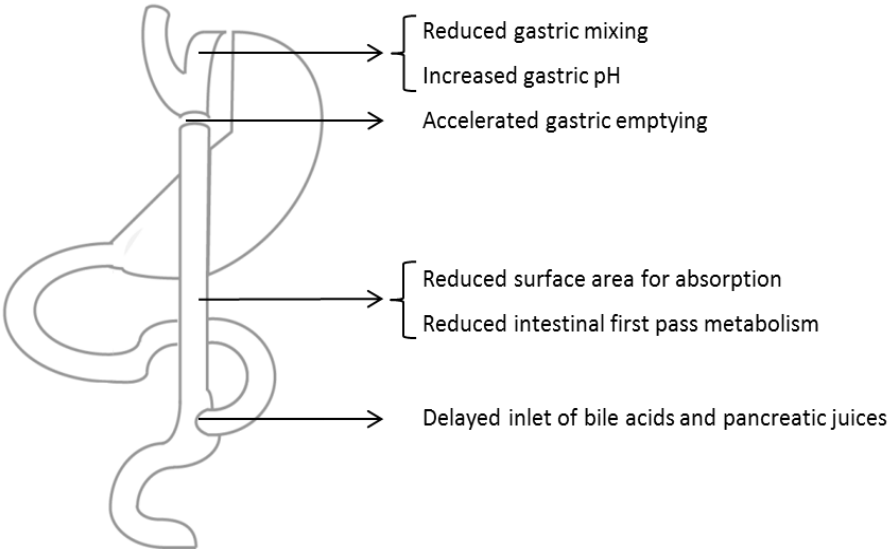

Figure 3: Influence of RYGB on different factors influencing oral drug bioavailability

GASTRIC MIXING

Gastric mixing promotes disintegration, which is required for drug absorption. Gastric mixing, and thus disintegration, may be reduced after RYGB as the stomach volume is reduced after RYGB [58]. To avoid the need for disintegration, drugs can be administered as a liquid formulation, crushing/chewing solid formulation or as an orodispersible formulation, if this is available for the concerning drug.

GASTRIC ACID SECRETION AND PH

A small gastric pouch, which is separated from the native stomach, is created in patients with a gastric bypass. In the study of Smith CD et al. (patients with RYGB: n=10; controls: n=15), the gastric acid secretion from the gastric pouch was markedly reduced compared to the gastric acid secretion from the total stomach of age- and sex-matched control subjects [31]. This was the case for both the basal secretion (mean±SEM: 0.01 ± 0.01 mEq/hr post-RYGB vs. 4.97 ± 0.66 mEq/hr in the control group) and the pentagastrin-based stimulated secretion (0.08 ± 0.04 mEq/hr post-RYGB vs. 12.11 ± 1.34 mEq/hr in the control group) [31]. These findings were confirmed by Behrns KE et al. [32], indicating that the gastric acid secretion after gastric bypass is negligible as the majority of the parietal cells (i.e. acid-producing cells) are bypassed. This results in an elevated gastric pH, which will

have an impact on the solubility of drugs as it influences the ionization [59]. Basic drugs will have a decreased solubility after RYGB as there is less ionization, and acidic drugs will have an increased solubility as there is more ionization. In modelling studies, an average pH of 6.6 was set up to simulate the gastric pouch after RYGB in fasted state [60;61]. An *in vitro* analysis of the dissolution of psychiatric medications after RYGB showed that the dissolution of 10 out of 22 drugs was significantly reduced, while 2 drugs had significantly greater dissolution in a post-RYGB environment in comparison with a control environment [61]. The level of dissolution gives no direct information on the therapeutic effect of drugs, but it provides already an indication of the level of absorption since the absorption of drugs is often limited by the dissolution rate.

GASTRIC EMPTYING

Gastric content, hormones and neural influences regulate gastric emptying. It has been shown by different methods such as a D-xylose test, a paracetamol test and scintigraphic measurements that gastric emptying for liquids is accelerated after RYGB [18;62-65]. Oral administration of a liquid drug may therefore result in faster absorption and in a shorter time to reach maximal plasma concentration (T_{max}). The influence of RYGB on gastric emptying for solids is more controversial, as Horowitz M et al. have shown that the gastric emptying for solids is slower postoperatively, while Dirksen C et al. have shown that it is faster [63;65].

BILE SALT SOLUBILIZATION AND ENTEROHEPATIC RECIRCULATION

Roux-en-Y gastric bypass is associated with alterations of the anatomical structure of the upper intestinal tract, which could affect the enterohepatic recirculation of bile acids. The inlet of bile acids and pancreatic juices in the small intestine is delayed, thus the interaction between these compounds and food/drugs is delayed [12]. These changes have mainly an impact on lipophilic drugs as these are dependent on bile acids for their dissolution/solubility. Furthermore, lipophilic drugs often undergo enterohepatic recirculation, influencing its steady-state concentration [58].

Patti et al. showed in individuals who had undergone a RYGB 2-4 years before (n=9), that the serum concentration of bile acids was more than twofold higher than in overweight (n=10) or severely obese individuals (n=5) without bariatric surgery [66]. This was the same for the subfractions taurodeoxycholic, glycocholic, glycochenodeoxycholic, and glycodeoxycholic acids. This has been confirmed by Simonen et al. (n=30) [67], who have also shown that the total serum bile acid concentration in fasted state was twofold increased post-RYGB. However, the increased secretion of bile acids may not be sufficient to compensate the delayed inlet as it has been shown that the absorption of fat is reduced after RYGB, as mentioned before [17;20;21].

INTESTINAL TRANSIT TIME

Dirksen et al. have studied the small intestinal transit time after a meal by a scintigraphic technique in 17 patients who had undergone a RGYB more than a year ago and in 9 healthy control subjects. They have shown that the small intestinal transit time was longer in patients with RYGB compared to control subjects [65]. This does not entirely correspond with the findings of Morinigo et al., who have shown that the oro-caecal transit time was shorter in RYGB-patients by performing a lactulose breath test. However, the oro-caecal transit includes pouch emptying and small intestinal transit, so it reflects not only the intestinal transit time [64]. Carswell et al. have also measured the oro-caecal transit time using sulphasalazine. In this study RYGB had no impact on the oro-caecal transit time [21]. Dirksen et al. have also determined the colonic transit time, which was unaltered after RYGB [65].

SURFACE AREA FOR ABSORPTION

By performing a RYGB, a part of the stomach and the proximal small intestine is bypassed resulting in a reduction of the functional gastrointestinal length [12]. Moreover, the proximal small intestine has the largest surface area of the gastrointestinal tract by the presence of villi and microvilli. A bypass of the duodenum and the proximal part of the jejunum results in a large reduction of the surface area for absorption [57;68]. This can have an impact on the bioavailability of drugs, especially since most of the orally administered drugs are maximally absorbed in the small intestine [58].

Besides, Spak et al. have shown that the appearance of the mucosa of the Roux-limb is altered 6-8 months after the surgery [68]. They showed that the height of the villi was decreased after RYGB, which also contributes to the reduction of the surface area. However, cell proliferation was increased, which might be a compensatory mechanism for the loss of cells at the tips of the villi.

FIRST PASS METABOLISM AND EFFLUX IN THE INTESTINE

Drug metabolism and drug efflux at the level of the intestinal wall are factors that affect the bioavailability of drugs too. The influence of these variables may vary between drugs and over different parts of the intestine [58]. The expression of the efflux transporter P-glycoprotein increases from the proximal to the distal small intestine, while the expression of cytochrome P450 enzymes is the highest in the duodenum and jejunum and decreases to more distal sites [69]. Bypassing parts of the small intestine with a high abundance of CYP enzymes can therefore lead to alterations in oral drug bioavailability as there will be less metabolism and may increase the relative influence of P-glycoprotein.

RECEPTORS AND TRANSPORTERS

The majority of drugs, provided that they are non-ionized, enter into the systemic circulation by passive diffusion from the gastrointestinal tract. Ionized drugs require active transport for absorption. However, receptors and transporters are not homogenously distributed through the small intestine; hence, bypassing the proximal part of the small intestine can be associated with bypassing receptors. This can influence the bioavailability of drugs that need these receptors for absorption [70].

1.5.2 Overview of studies/case reports regarding influence of RYGB on bioavailability of drugs

In Table 4-12, an overview of pharmacokinetic studies and case-reports that illustrate the influence of RYGB on oral drug bioavailability, is shown. The studies have been grouped by ATC-class concerned. The results demonstrate that the influence of RYGB is drug dependent as the

bioavailability can be increased, decreased or it can also remain the same. The observed drug-dependent effect may be related to drug specific properties as permeability and solubility, but also to drug-specific dependence on gastrointestinal drug metabolizing enzymes and efflux transporters [71]. While searching for evidence, we found that a systematic approach to examine the influence of RYGB on drug bioavailability is lacking. Furthermore, there are many differences between the studies, including differences in design and in time after surgery at which analyses are performed, which makes it difficult to compare the results.

Table 4: Overview of reported human pharmacokinetic (PK) studies and case-reports (CR) regarding oral drug bioavailability after RYGB - ATC class A

Drug	Reference	Study type	Number of subjects	Time points studied	Effect on pharmacokinetics	Explanation / implications for practice
Metformin (2 tablets of 500 mg metformin)	Padwal et al. [72]	PK	16 patients post-RYGB (13♀, 3♂); 16 sex- and BMI-matched controls	≥ 3 months post-RYGB	↑ $AUC_{0-\infty}$ post-RYGB (N.S.) Significant ↑ bioavailability post-RYGB	Possible mechanisms for the increased absorption: - A prolonged intestinal transit time can increase the absorption, as metformin is mainly absorbed in the small intestine and a permeability rate limited drug - Metformin is absorbed by transcellular transport depending on organic cation transporters, and mostly by paracellular transport; there might be an upregulation of these transporters after surgery - Small intestinal adaptation post-RYGB might occur
Omeprazole (20 mg)	Tandra et al. [73]	PK	18 RYGB patients; 18 sex-, BMI-, race- and age- matched controls	At least 1 year post-RYGB 5 drugs given as a "cocktail"	Omeprazole = probe for CYP2C19 = $AUC_{5\text{-}OH\text{-}omeprazole}/AUC_{omeprazole}$ = C_{max} Significant ↓ T_{max}	CYP2C19 activity was similar in the post-RYGB group and in the control group, which was not surprising as the expression of CYP1A2 in the gut wall is not clinically significant. The shorter T_{max} might be explained by the faster gastric emptying.
Tolbutamide (100 mg)	Tandra et al. [73]	PK	18 RYGB patients; 18 sex-, BMI-, race- and age- matched controls	At least 1 year post-RYGB 5 drugs given as a "cocktail"	Tolbutamide = probe for CYP2C9 = $AUC_{1\text{-}OH\text{-}tolbutamide}/AUC_{tolbutamide}$ = C_{max} Significant ↓ T_{max}	CYP2C9 activity was similar in the post-RYGB group and in the control group, which was not surprising as the expression of CYP1A2 in the gut wall is not clinically significant. The shorter T_{max} might be explained by the faster gastric emptying.

Table 5: Overview of reported human pharmacokinetic (PK) studies and case-reports (CR) regarding oral drug bioavailability after RYGB - ATC class B

Drug	Reference	Study type	Number of subjects	Time points studied	Effect on pharmacokinetics	Explanation / implications for practice
Rivaroxaban	Mahlmann et al. [74]	CR	1 patient (♀)	Initiation of 20 mg of rivaroxaban 3 months post-RYGB (=standard dosage)	After the first administration: C_{max} of 224.22 ng/mL was reached after 3 h. The concentration decreased slowly until trough level of 35.54 ng/mL after 24 h. After the second administration C_{max} of 262.46 ng/mL was reached. There was an immediate effect on INR.	This case suggests that rivaroxaban is immediately absorbed after oral administration and no dose adjustments for rivaroxaban seem to be necessary after RYGB.
Warfarin	Sobieraj et al. [75]	CR	1 patient (♀)	Before RYGB: warfarin Time of admission: heparin and then enoxaparin After RYGB: restarting warfarin	3 months post-RYGB: INR of 1.30 with a standard dose of warfarin and good adherence. Patient required dosages up to 20 mg/day to obtain a therapeutic INR.	Resistance to warfarin therapy is possible after RYGB. After RYGB: close monitoring and/or dose adjustments for warfarin can be necessary to obtain therapeutic anticoagulation.

Table 6: Overview of reported human pharmacokinetic (PK) studies and case-reports (CR) regarding oral drug bioavailability after RYGB - ATC class C

Drug	Reference	Study type	Number of subjects	Time points studied	Effect on pharmacokinetics	Explanation / implications for practice
Atorvastatin (20-80 mg)	Skottheim et al. [76]	PK	12 patients	Before surgery Range 3-6 weeks post-RYGB	Altered AUC: range from a 2.9 times decrease to a 2.3 times increase in AUC. Patients with a large AUC_{0-8h} before RYGB, had a reduced AUC_{0-8h} post-RYGB. Patients with a lower AUC_{0-8h} before RYGB, had an increased AUC_{0-8h} post-RYGB.	The influence of RYGB on the bioavailability of atorvastatin depends on the first-pass metabolic capacity before surgery, as atorvastatin is a substrate of CYP3A4 and CYP3A5 and it is also a P-gp substrate.
Furosemide	Tandra et al. [73]	PK	13 patients post-RYGB, 14 sex-, BMI-, race- and age- matched controls	At least 1 year post-RYGB	Significant ↓ T_{max} 1h and 2h after oral administration: significant higher concentration post-RYGB No significant differences at time point 1.5; 2.5; 4 and 8h	The shorter T_{max} might be explained by the faster emptying of the small gastric pouch. The faster absorption of furosemide after RYGB is correlated with an earlier urinary sodium excretion.

Table 7: Overview of reported human pharmacokinetic (PK) studies and case-reports (CR) regarding oral drug bioavailability after RYGB - ATC class G

Drug	Reference	Study type	Number of subjects	Time points studied	Effect on pharmacokinetics	Explanation / implications for practice
Etonorgestrel (ENG) (implant containing 68 mg of ENG - Implanon®; inserted 1-2 months prior to RYGB)	Ciangura et al. [77]	CR	3 patients (3♀)	Before surgery 3 months post-RYGB 6 months post-RYGB	Serum ENG concentrations: progressively ↓ post-RYGB, but sufficient to maintain a contraceptive effect. Patient 1: ENG concentration 9 months after insertion was comparable to the lower range observed 3 years after insertion in normal-weight women. Patient 2 and 3: ENG concentration 6 months after insertion was comparable to the concentration 8 months after insertion in normal-weight women.	The increased fat mass is associated with an increased volume of distribution, which may explain these results. Furthermore, there is a wide variation in ENG clearance and a lot of co-variants can influence drug elimination; possibly, patient 1 presents many of these factors. These results suggest that Implanon® is a safe contraceptive method after RYGB, but the large variation in ENG concentration between patients need to be taken into account.

Table 8: Overview of reported human pharmacokinetic (PK) studies and case-reports (CR) regarding oral drug bioavailability after RYGB - ATC class H

Drug	Reference	Study type	Number of subjects	Time points studied	Effect on pharmacokinetics	Explanation / implications for practice
Levothyroxin (LT4) (600 µg of oral LT4 tablets)	Rubio et al. [78]	PK	15 patients post-RYGB (13♀, 2♂); 15 controls (12 ♀, 3♂; no match)	2-3 months post-RYGB	No significant differences in plasma levels of total T4 and serum-free T4 between both groups at any time point. Significant ↑ T_{max} of total T4 post-RYGB.	There was no reduced LT4 absorption post-RYGB. However, a small, significant delayed absorption post-RYGB was observed.
Levothyroxin (solution of 600 µg of LT4)	Gkotsina et al. [79]	PK	7 patients	Before surgery 35 days post-RYGB	= $AUC_{0\text{-}4h}$ post-RYGB = C_{max} post-RYGB = T_{max} post-RYGB $AUC_{0\text{-}4h}$ and C_{max} had the tendency to be increased after RYGB.	The absorption of LT4 was not reduced after RYGB.
Levothyroxin (replacement of LT4 tablet to liquid formulation – same dosage)	Pirola et al. [80]	CR	4 patients (4♀)	Before surgery and until 1 year post-RYGB: tablet 1 year post-RYGB: switch of formulation: from tablet to liquid formulation	All patients were euthyroid before RYGB. One year post-RYGB: subclinical hypothyroidism regarding the thyroid hormone profile => alteration of formulation When re-administration of tablet instead of liquid formulation: serum TSH concentration increased again, which suggest malabsorption of LT4 from the tablets.	Decrease in serum TSH levels after replacement of LT4 tablet to liquid formulation. So, there is higher absorption of LT4 from a liquid formulation than from a tablet. Therefore, it could be interesting to switch to a liquid formulation as patients after RYGB have an impaired LT4 absorption from tablets.
Prednisone (10 mg/day)	Aron-Wisnewksy et al.[81]	CR	1 patient	Before RYGB 1 month post-RYGB 12 months post-RYGB 15 months post-RYGB	Prednisolone (active metabolite of prednisone) 1 month post-RYGB: ↓ $AUC_{0\text{-}24h}$ (1.90x), ↓ C_{max} (1.75x), ↑ T_{max} 12 months vs 1 month post-RYGB: ↑ $AUC_{0\text{-}24h}$, ↑ $C_{max;}$; tended to return to baseline levels	Prednisone is a substrate of intestinal P-gp; one month post-RYGB the relative influence of P-gp (highest expression in the distal part of the digestive tract) can be maximized as the proximal part of the small intestine is bypassed, resulting in a decreased bioavailability. One year post-RYGB, the bioavailability increases again, which may suggest an adaptation of the P-gp expression over time. This case suggests that intestinal adaptation after RYGB occur as there was a large variation of plasma concentrations over time.

Table 9: Overview of reported human pharmacokinetic (PK) studies and case-reports (CR) regarding oral drug bioavailability after RYGB - ATC class J

Drug	Reference	Study type	Number of subjects	Time points studied	Effect on pharmacokinetics	Explanation / implications for practice
Amoxicillin, nitrofurantoin, amoxicillin/clavulate	Magee et al. [82]	CR	1 patient (pregnant ♀ with urinary tract infection)		Initiation of oral amoxicillin; 2 days later: > 100 000 colonies *E. Coli* (sensitive to amoxicillin). Initiation of oral nitrofurantoin; 2 days later: > 100 000 colonies *E. Coli* (sensitive to nitrofurantoin). Failure of two oral antibiotics: prophylaxis with amoxicillin/clavulanate (500 mg/day); after 10 days: 2nd case of pyelonephritis. ⇨ Treatment: intravenous ceftriaxone (48 h)	No plasma levels of the antibiotics were checked in this patient, so there is no information about the influence of RYGB on the absorption of these drugs. However, clinical effect was lacking in this patient post-RYGB, so after RYGB: monitoring should be considered.
Azithromycin (2 tablets of 250 mg azithromycin)	Padwal et al. [83]	PK	14 patients post-RYGB (14♀); 14 sex- and BMI-matched controls	≥ 3 months post-RYGB	Significant ↓ $AUC_{0\text{-}24h}$ post-RYGB ↓ C_{max} post-RYGB (p=0.08)	The most important absorption site for azithromycin is the upper gut, which is bypassed after RYGB; furthermore azithromycin is a target for numerous membrane transporters, of which the expression might be changed post-RYGB. After RYGB: clinical monitoring and/or dose adjustments for azithromycin should be considered.
Erythromycin (1x 250 mg of erythromycin)	Prince et al. [84]	CR	1 patient (♀)		↑ $AUC_{0\text{-}12h}$ ↓ C_{max} ↑ T_{max}	There is a delay in absorption of erythromycin after RYGB.
Isavuconazole (day 1-3: loading dose with 200 mg 3x/day; subsequently 200 mg 1x/day)	Knoll et al. [85]	CR	1 patient	6 years post-RYGB	After 7 days of treatment: below the trough level; afterwards, the dose was increased to 200 mg every 12h and the target concentration was reached.	The authors assumed that the shorter intestinal transit time caused the reduction in bioavailability. Isavuconazole can be effective in RYGB-patients, but higher dosages may be required; monitoring should be considered.
Linezolid (600 mg of linezolid)	Hamilton et al. [86]	PK	5 patients (1♀, 4♂)	Before RYGB 3 months post-RYGB	Significant ↑ $AUC_{0\text{-}\infty}$ post-RYGB	After RYGB, a reduction in clearance of linezolid was observed in all 5 patients with weight loss. The exposure of linezolid is lower in obese vs. non-obese subjects, so higher doses are necessary in obese people.

Moxifloxacine (400 mg of moxifloxacin iv and 400 mg moxifloxacin tablet; 1 week washout)	De Smet et al. [87]	PK	12 patients post-RYGB (8♀, 4♂)	≥ 6 months post-RYGB	Oral bioavailability after RYGB was almost complete (AUC_{∞},oral/AUC_{∞},iv=88%). After oral administration: C_{max} was lower than with iv administration.	The enterohepatic recirculation might be higher after a gastric bypass. No dose adjustments for moxifloxacin are needed after RYGB.
Anti-HIV therapy	MacBrayne et al. [88]	CR	1 patient (♂)	Initiation of 300 mg of tenofovir disoproxil fumarate (TDF) once daily, 200 mg of emtricitabine (FTC) once daily and 600/100 mg of darunavir/ritonavir (DRV/r) twice daily 24 months post-RYGB	17 days after initiation of therapy: observed trough levels of the different drugs were comparable to historic data.	Other HIV treating drugs are dependent on gastric acid for its absorption, and the T_{max} is also very important as the shorter T_{max}, the less time contact between the drug and the gut is necessary. Therefore, they have chosen for this HIV treatment. In this case, trough concentrations of TDF/FTC (1x/day) and DRV/r (2x/day) were similar to historic data.

Table 10: Overview of reported human pharmacokinetic (PK) studies and case-reports (CR) regarding oral drug bioavailability after RYGB - ATC class L

Drug	Reference	Study type	Number of subjects	Time points studied	Effect on pharmacokinetics	Explanation / implications for practice
Cylcosporin A	Marterre et al. [89]	PK	3 patients		Significant ↑ in weight adjusted cyclosporin A requirements post-RYGB. ↑ in absolute requirements of cylcosporin A	Higher dosages of cyclosporin A are required in patients post-RYGB to obtain similar exposure as in patients without bariatric surgery; monitoring should be considered post-RYGB.
Methotrexate (MTX; 15 mg weekly)	Aron-Wisnewksy et al. [81]	CR	1 patient	Before RYGB 1 month post-RYGB 12 months post-RYGB 15 months post-RYGB	1 month post-RYGB: = AUC_{0-24h} , = C_{max} , = T_{max} 12 and 15 months post-RYGB: MTX plasma levels decreased dramatically (switch from oral to subcutaneous treatment was necessary)	Differences in efficacy between oral and subcutaneous administration, might explain an incomplete absorption after oral administration. The absorption of MTX decreases with an increased pH and the expression of H^+/co-transporter, the most important absorption route of MTX, is the highest in the duodenum. This can explain the reduced absorption 1 year post-RYGB, but not the lag time for this enormous decrease.
Mycophenolate mofetil (MMF) (1 g BID; except 1 post-transplant patient: 750 mg BID)	Rogers et al. [90]	PK	4 ESRD patients (pre-transplant) and 2 post-transplant patients	Different time after RYGB (ranging from 0.16 – 7.41 years)	Comparison with historical records: ↓ $AUC_{0-\infty}$ post-RYGB ↓ C_{max} post-RYGB (No statistical tests performed)	Higher dosages of mycophenolic acid might be necessary in post-RYGB patients to obtain similar exposure as in patients without bariatric surgery; monitoring should be considered post-RYGB.
Sirolimus (SRL) (6 mg of sirolimus)	Rogers et al. [90]	PK	4 ESRD patients (pre-transplant)	Different time after RYGB (ranging from 0.16 – 3.50 years)	Comparison with historical records: Trend to decreased AUC levels post-RYGB (No statistical tests performed)	Higher dosages of sirolimus might be necessary in post-RYGB patients to obtain similar exposure as in patients without bariatric surgery; monitoring should be considered post-RYGB.
Tacrolimus (FK) (4 mg BID)	Rogers et al. [90]	PK	4 End Stage Renal Disease (ESRD) patients (pre-transplant) and 1 post-transplant patient	Different time after RYGB (ranging from 0.16 – 7.41 years)	Comparison with historical records: ↓ AUC_{0-12h} post-RYGB ↓ C_{max} post-RYGB ↓ T_{max} post-RYGB AUC/dose ratio was lower than in patients without surgery. (No statistical tests performed)	Large interindividual variability, which can be explained by modifications of the small intestine post-RYGB regarding absorption (tacrolimus is metabolized by CYP 3A4/5 and a substrate for P-gP) Higher dosages of tacrolimus might be necessary in post-RYGB patients to obtain similar exposure as patients without bariatric surgery; monitoring should be considered post-RYGB.
Tamoxifen	Wills et al. [91]	CR	3 patients (♀)		All 3 women: subtherapeutic steady state levels of tamoxifen with standard dose; in 1 ♀: the dose was doubled (2 x 20 mg/day) and then therapeutic levels were reached.	After RYGB, steady-state concentrations of tamoxifen could be reduced. After RYGB: close monitoring of tamoxifen levels; if malabsorption: parenteral alternatives should be considered.
Temozolomide (190 mg of temozolomide = 75 mg/m²)	Park et al. [92]	CR	1 patient (♂)	Initiation 4 months post-RYGB	The PK parameters were comparable with previously reported levels in literature	The PK of oral temozolomide seemed not to be affected by the increased gastric pH after RYGB, even though temozolomide is stable in acidic environment and labil in neutral and basic environments.

Table 11: Overview of reported human pharmacokinetic (PK) studies and case-reports (CR) regarding oral drug bioavailability after RYGB - ATC class N

Drug	Reference	Study type	Number of subjects	Time points studied	Effect on pharmacokinetics	Explanation / implications for practice
Caffeine (40 mg)	Tandra et al. [73]	PK	18 RYGB patients, 18 sex-, BMI-, race- and age- matched controls	At least 1 year post-RYGB 5 drugs given as a "cocktail"	Caffeine = probe for CYP1A2 = $AUC_{paraxanthine}/AUC_{caffeine}$ Significant ↓ T_{max} Significant ↑ C_{max}	CYP1A2 activity was similar in the post-RYGB group than in the control group, which was not suprising as the expression of CYP1A2 in the gut wall is not clinically significant. The shorter T_{max} might be explained by the faster emptying of the small gastric pouch.
Duloxetine [93] (60 mg of duloxetine)	Roerig et al. [33]	PK	10 patients post-RYGB; 10 sex-, BMI- and age-matched controls	9 – 15 months post-RYGB	Significant ↓ $AUC_{0-\infty}$ post-RYGB Significant ↓ T_{max} post-RYGB No significant difference in C_{max}	The most important absorption site for duloxetine is the duodenum; the decreased absorption might be explained by the reduction in absorptive surface area. After RYGB: clinical monitoring should be considered.
Midazolam (1 mg)	Tandra et al. [73]	PK	18 RYGB patients, 18 sex-, BMI-, race- and age- matched controls	At least 1 year post-RYGB 5 drugs given as a "cocktail"	Midazolam = probe for CYP3A Post-RYGB: = AUC_{0-6h} = C_{max} Significant ↓ T_{max}	CYP3A activity was similar in the post-RYGB group and in the control group. This was not expected as it has already been shown that 50% of the activity of CYP3A is contributed by the gut wall, so intestinal adaptation after RYGB is possible. The shorter T_{max} might be explained by the faster emptying of the small gastric pouch.
Morphine (5 mL of a solution with 30 mg of morphine sulfate)	Lloret-Linaeres et al. [94]	PK	30 patients (24♀, 6♂) (visit 2: 24 patients; visit 3: 25 patients)	Before RYGB (visit 1) 7-15 days post-RYGB (visit 2) 6 months post-RYGB (visit 3)	Visit 2 and 3 vs visit 1: Significant ↑ AUC_{0-12h} Significant ↑ C_{max} Significant ↓ T_{max}	Changes in bioavailability due to weight loss and RYGB surgery. The administrated dose of morphine after RYGB should be lower than before surgery.
Phenytoin	Pournaras et al. [95]	CR	1 patient (♂)	1 year post-RYGB: 300 mg of phenytoin was added to his treatment of epilepsy (60 mg of phenobarbitone) as he had a seizure; 2 years post-RYGB he had a seizure again.	2 years post-RYGB: levels of phenytoin were undetectable and those of phenobarbitone were subtherapeutic; then the dosage of phenytoin was increased up to 500 mg/day. No effect on the phenytoin levels was observed.	The low levels of phenytoin post-RYGB may be explained by the reduced surface area for absorption, especially the exclusion of the duodenum and proximal jejunum. Drugs with a narrow therapeutic window need to be prescribed with caution to patients with RYGB. If prescribed after RYGB, close monitoring is essential to prevent under- or overdosing.

Serotonin Reuptake Inhibitors	Hamad et al. [96]	PK	12 patients (11♀, 1♂)	Before surgery 1 month post-RYGB 6 months post-RYGB 12 months post-RYGB	In 8/12 patients: ↓$AUC_{0\text{-}7h}$ one month post-RYGB; in 6/8 patients $AUC_{0\text{-}7h}$ levels returned to baseline values 6 months post-RYGB. In 3/12 patients: $AUC_{0\text{-}7h}$ levels remained constant or increased post-RYGB. In 1/12 patients: $AUC_{0\text{-}7h}$ was the same one month post-RYGB, but decreased gradually until 1 year post-RYGB .	The disadvantage of this study is that there was variability in SRI medication among the patients. One month post-RYGB, the patients were at risk for a lower SRI bioavailability. This was more prominent in the patients taken SSRI than the patients taken SNRI, which might be explained by different drug dissolution properties as the SSRIs were significantly less soluble in a simulated post-RYGB solution than in a pre-RYGB solution. In most of the patients, the levels returned to baseline values 6 months post-RYGB, which could be explained by an intestinal adaptation to increase the absorptive surface area post-RYGB, resulting in an improved absorption. After RYGB: psychiatric monitoring should be considered.
Sertraline (100 mg of sertraline)	Roerig et al. [97]	PK	5 patients post-RYGB; 5 sex-, BMI- and age-matched controls	9-15 months post-RYGB	Significant ↓ $AUC_{0\text{-}10.5h}$ post-RYGB Significant ↓ C_{max} post-RYGB	The mean disposition of sertraline was smaller post-RYGB, which can be explained by the bypass of the proximal part of the small intestine, which is the main absorption site for sertraline.

Table 12: Overview of reported human pharmacokinetic (PK) studies and case-reports (CR) regarding oral drug bioavailability after RYGB - ATC class P

Drug	**Reference**	**Study type**	**Number of subjects**	**Time points studied**	**Effect on pharmacokinetics**	**Explanation / implications for practice**
Hydroxychloroquine (HCQ; 400 mg/day)	Aron-Wisnewksy et al. [81]	CR	1 patient	Before RYGB 1 month post-RYGB 12 months post-RYGB 15 months post-RYGB	1 month post-RYGB: ↑ $AUC_{0\text{-}24h}$ (2x), ↑ C_{max} (3.5x) 12 and 15 months post-RYGB: progressive decrease of $AUC_{0\text{-}24h}$ and C_{max}, reaching baseline values	HCQ is a basic drug; after RYGB the gastric pH is increased, which can result in a higher solubility of HCQ and subsequently a better absorption resulting in higher plasma levels of HCQ. The return to baseline levels one year post-RYGB can be explained by some degree of gastric pouch enlargement over time, associated with a decrease in gastric pH.

CHAPTER 2: OBJECTIVES, HYPOTHESES AND DESIGN

2 OBJECTIVES, HYPOTHESES AND DESIGN

2.1 Research objectives

The overall aim of this project was to understand the effect of Roux-en-Y gastric bypass on food intake, medication/supplement use and oral drug disposition in order to develop pharmacotherapeutic and dietary guidelines.

To attain this overall aim, the following specific objectives have been designed:

- Examine the changes in dietary pattern and body composition before and after RYGB
- Explore the use of medication and supplements before and after RYGB
- Evaluate the disposition of orally administered drugs in patients pre- and post-RYGB using model compounds, which have specific absorption characteristics
- Evaluate the influence of RYGB on the efficacy of controlled release formulations
- Validate a physiologically-based pharmacokinetic model for predicting oral bioavailability following RYGB
- Get insight in the current care for bariatric patients before and after surgery

2.2 Hypotheses

The following hypotheses were drafted:

1) Nutrient intake improves after RYGB, but the intake of essential nutrients remains inadequate and contributes to the development of micronutrient deficiencies.
2) The extent to which RYGB has an impact on oral drug disposition is determined by the characteristics of the drug (solubility and permeability).
3) Current care of bariatric patients differs widely between Flemish hospitals.

2.3 Design of the PhD project

To achieve these objectives and test the hypotheses, different studies were conducted. Figure 4 provides an overview of the different parts of the project.

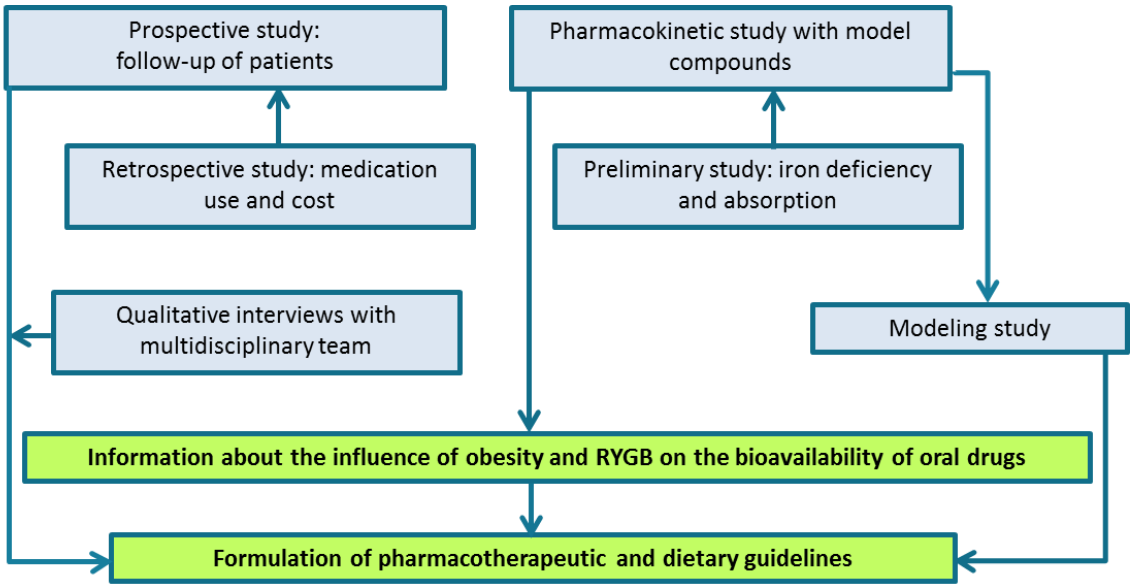

Figure 4: Overall design of the research project

A major part of the PhD project consisted of a prospective study in which patients with a planned RYGB were followed until one year post-RYGB. We collected data about their food intake, body composition, medication and supplement use and clinical parameters (Chapter 3 and 4).

To evaluate the influence of RYGB on disposition of micronutrients and drugs, different studies were planned. First, a preliminary study regarding iron deficiency and its absorption was performed (Chapter 5). Later, a more extensive pharmacokinetic study with iron gluconate was carried out (Chapter 6). We have also performed pharmacokinetic studies with different model compounds: metoprolol, fenofibrate and posaconazole. Moreover, for metoprolol we used two different formulations: an immediate release and a controlled release formulation. The results from the pharmacokinetic studies are shown in Chapter 6, 7 and 8. Subsequently, the results for metoprolol from our PK-study were compared with results from a physiologically-based pharmacokinetic modelling and simulation. This last part of the study was performed in collaboration with Simcyp and is reported in Chapter 7.

To get insight in the influence of RYGB on medication use and the associated costs, a retrospective study was performed. In this study data about medication use and the associated costs were collected before RYGB and up to 4 years after RYGB (Chapter 9). Furthermore, to get insight in the current clinical practice, we performed a qualitative study in 12 Flemish hospitals by interviewing health care professionals (HCPs), involved in screening and follow-up of bariatric patients, regarding medication adjustments, nutritional deficiencies and multidisciplinary approach (Chapter 10).

PART II: INFLUENCE OF RYGB ON INTAKE OF MACRO- AND MICRONUTRIENTS

CHAPTER 3: MACRONUTRIENT INTAKE AND THE ASSOCIATION WITH BODY COMPOSITION IN RYGB PATIENTS BEFORE AND AFTER SURGERY

Manuscript in preparation

3 MACRONUTRIENT INTAKE AND THE ASSOCIATION WITH BODY COMPOSITION IN RYGB PATIENTS BEFORE AND AFTER SURGERY

Background: Roux-en-Y gastric bypass (RYGB) is an effective treatment for morbid obesity through a reduction of the gastric capacity and a bypass of the duodenum and proximal jejunum, resulting in restricted food intake and malabsorption. The objective of this study was (1) to evaluate the usual intake of energy and macronutrients in patients before and after RYGB; and (2) to evaluate associations between intake of different macronutrients and body composition.

Methods: Patients who had planned RYGB surgery were included at University Hospitals Leuven, Belgium and were followed until 12 months post-RYGB. Patients completed an estimated dietary record of two non-consecutive days before and 1, 3, 6 and 12 months post-RYGB. Usual dietary intake of energy and different macronutrients (including mono- & disaccharides, polysaccharides, and different types of fatty acids) was calculated. Body composition was estimated by a bioelectrical impedance analysis (BIA) and fat mass index (FMI) and fat free mass index (FFMI) were calculated. Linear mixed models for repeated measures were used for analysis; statistical significance was set at $p<0.05$.

Results: Fifty-four patients (33♀, 21♂; mean age: 48.0 [95% CI 46.6; 49.3] years; mean preoperative BMI: 40.4 [95% CI 39.4; 41.4] kg/m²) were included. One month post-RYGB, the usual dietary intake of energy and macronutrients was significantly decreased compared to pre-RYGB, but gradually increased until 12 months post-RYGB, remaining below baseline values. A significant increase in EN% from proteins was observed and a decrease for the EN% from fat. Almost all patients had an inadequate fiber intake at all time points. FMI significantly decreased until 1 year post-RYGB and FFMI until 6 months post-RYGB. A significantly, positive correlation between FFMI and the intake of proteins was observed ($p<0.01$).

Conclusions: Protein intake after RYGB needs to be supported as protein intake is correlated with preservation of FFMI. This trial was registered at clinicaltrials.gov as NCT01571180.

3.1 Introduction

There is an increased demand for bariatric surgery as the prevalence of obesity has reached epidemic proportions [1]. Nowadays, bariatric surgery is considered as the only efficient way, in combination with lifestyle interventions, to achieve major and sustainable weight reduction [98]. The bariatric procedure most commonly performed is a Roux-en-Y gastric bypass (RYGB). A RYGB is characterized by a reduction of the gastric capacity and a bypass of the duodenum and proximal jejunum, resulting in restricted food intake and malabsorption [12].

The objective of bariatric surgery is losing weight, preferably in adipose tissue; however, patients with bariatric surgery also lose fat free mass (FFM) [99]. These body composition changes can be influenced by the type and amount of food consumed. Therefore, some recommendations regarding nutritional follow-up after RYGB have already been designed. For instance, a daily protein intake of 60-120 g is recommended as a positive association between a high protein intake and preservation of lean body mass in bariatric patients has been demonstrated [44;100]. In this specific population group, however, dietary recommendations have not yet been developed for all nutrients.

Previous studies have demonstrated an enormous reduction in energy intake after RYGB, which is due to a reduction in carbohydrate, protein and fat intake [17;28;40-42]. The energy intake increases again with time after surgery, but the energy and macronutrient intake remained below baseline values until 5 years post-RYGB [42]. Moizé et al. have also shown that the energy intake from the different macronutrients was not significantly different between patients 5 years after RYGB and baseline [42]. In previous studies, the contribution of the various macronutrients was investigated with a focus on total intake, while no detailed information about subtypes of the macronutrients was provided. Furthermore, the studies that have been performed, focused on either body composition or food intake, while the interaction between both is important.

Therefore, the objective of the current study was (1) to study the evolution of energy intake and the contribution of the different macronutrients in patients pre- and post-RYGB; (2) to investigate the

intake of mono- & disaccharides and polysaccharides and of the different types of fatty acids; and (3) to analyze if the association between the intake of the different macronutrients and body composition.

3.2 Methods

3.2.1 Selection of patients

Obese patients who had planned RYGB surgery between April 2012 and January 2014 at the University Hospitals Leuven, Belgium, were asked to participate in the study. If patients consented to participate, they were included in the study. Patients who had previously undergone bariatric surgery were not included in the study. Pregnant or lactating women were also not included. In all recruited patients, a laparoscopic RYGB with an alimentary limb of 120 cm and a small gastric pouch was performed by the same surgeon according the same procedure. The study was approved by the Medical Ethics Committee of the University Hospitals Leuven (ML8063) and was registered at Clinicaltrials.gov (NCT01571180). All patients gave written informed consent.

3.2.2 Study design and data collection

The current study is a prospective study, in which we have collected data before surgery and 1, 3, 6 and 12 months after RYGB during the standard follow-up consultations at University Hospitals Leuven, Belgium.

Weight was measured to the nearest 0.1 kg, with the subjects wearing indoor clothing with empty pockets and without shoes. BMI (kg/m²) was calculated by dividing the weight (kg) by the square of height (m²). Fat mass index (FMI) and fat free mass index (FFMI) were calculated by dividing the fat mass (kg) and fat free mass (kg), respectively, by the square of height (m²) [101]. The percentage of excess weight loss was calculated using the formula:

$$\%EWL = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight} - \text{ideal body weight}} \quad [102]$$

Patients were asked to keep a dietary record during two non-consecutive days preceding each consultation. In this semi-structured estimated dietary record, they noted all consumed foods and

beverages using estimated amounts. The completeness of the dietary records was verified on consultation, which was always done by the same person (IG). After completion, the diaries were processed into food quantities and codes by dietitians on the basis of a standard protocol, including a standard manual on food portions and household measures [103]. Based on this information, the actual intake of energy, proteins, fat (total fat, saturated fatty acids, mono- and polyunsaturated fatty acids) and carbohydrates (total carbohydrates, mono- & disaccharides, polysaccharides and fibers) was calculated using the Belgian Food Composition Database (FCDB) (NUBEL). If data were missing or incomplete in the NUBEL table, the Dutch (NEVO), Finnish (Fineli), and American (USDA) databases were used in the respective order. Based on the actual intake, we estimated the usual dietary intake of the studied micronutrients, using the Multiple Source Method (MSM) [104], which is a statistical method for the calculation of usual intake, based on short-term measurement data. Energy and macronutrient intake are always shown as usual intake. The energy percentage (EN%) of the different macronutrients were calculated by using the Atwater figures. The usual intake of carbohydrates was compared with the Institute of Medicine (IOM) age- and gender- specific Estimated Average Requirements (EAR) [105], and the usual intake of fiber and polyunsaturated fatty acids was compared to the adequate intake [105], to determine patients with an inadequate intake.

To estimate the body composition, a bioelectrical impedance analysis (BIA) was performed before and 3, 6 and 12 months post-RYGB. The BIA was performed with a Bodystat 1500 bioelectrical impedance body composition analyzer. In a subpopulation (n=21), Dual-energy X-ray absorptiometry (DXA) was also performed to estimate body composition before and 6 months post-RYGB. This allowed a comparison of BIA and DXA results, which fits within the cascade of validation procedures.

3.2.3 Internal validation of dietary records

We performed an internal validation to identify underreporting, using the Goldberg cut-off formulation:

$$EI:BMR = PAL$$

where EI stands for energy intake; BMR for basal metabolic rate (calculated by the James and Schofield formulation) [106] and PAL stands for physical activity level [107]. As PAL changes after surgery, the cut-off value to determine underreporting was set at 1.40 before and 6 and 12 months post-RYGB; at 1.0 one month post-RYGB and at 1.1 three months post-RYGB.

3.2.4 Statistical analysis

Linear models for repeated measurement were used to analyze evolution of dietary intake over time and to identify associations between energy/macronutrient intake and body composition. Time was modeled categorically, and an unstructured residual covariance matrix was modeled to account for intra-subject correlation. The Sidak multiple test correction was used. Data are shown as estimated means and 95% confidence intervals (95% CI), except otherwise mentioned. Statistical significance was set at $p<0.05$. The data were analyzed with SPSS Statistics 22.

3.3 Results

There were 54 participants (33♀, 21♂) included in this study with a mean age of 48 (95% CI 46.6; 49.3) years, and mean BMI of 40.4 (95% CI 39.4; 41.4) kg/m² before surgery. The %EWL was 70.6% (95% CI 66.5; 74.7) one year post-RYGB.

The total energy intake and absolute and relative intake of proteins, fatty acids and carbohydrates is shown in Table 13. The mean estimated usual energy intake was 2236 (95% CI 2053; 2418) kcal before surgery and decreased enormously one month after RYGB to 731 (95% CI 647; 816) kcal. After one month postoperative, the energy intake gradually increased again until one year post-RYGB to 1492 (95% CI 1388; 1596) kcal. The mean energy intake one year post-RYGB remained below baseline values. The same influence of RYGB was seen on the usual intake of proteins, fat (total fat, saturated fatty acids, mono- and polyunsaturated fatty acids) and carbohydrates (total carbohydrates, mono- & disaccharides, polysaccharides and fibers).

In Figure 5, the percentages of macronutrients contributing to the energy intake are shown at the different time points. Statistically significant differences in the percentages of the different macronutrients contributing to the energy intake were identified by comparing the different time points. Compared to baseline, there was a significant increase in EN% from proteins 1, 3 and 12 months post-RYGB, and at the same time points a significant decrease in EN% from fat. Regarding EN% of carbohydrates, no significant differences were observed. For alcohol, there was only a significant decrease 1 month post-RYGB compared to baseline. Compared to 1 month post-RYGB, the alcohol intake increased again 3 months (p=0.38), 6 months (p=0.06) and 12 months (p=0.06) post-RYGB.

Table 13: Estimated means (95% CI) of intake of energy, proteins, fat and carbohydrates and clinical parameters before and 1, 3, 6 and 12 months post-RYGB

	Before RYGB	**1 month post-RYGB**	**3 months post-RYGB**	**6 months post-RYGB**	**12 months post-RYGB**
Energy intake (kcal) [1-10]	2235.7 (2053.4; 2418.0)	731.3 (647.0; 815.6)	1084.8 (987.1; 1182.6	1301.7 (1204.6; 1398.9)	1491.9 (1387.5; 1596.4)
Protein intake (g) [1-4, 6-9]	91.5 (86.6; 96.4)	44.1 (37.6; 50.6)	52.8 (48.7; 56.8)	63.1 (57.2; 69.0)	68.8 (65.8; 71.8)
Fat intake (g) [1-7, 9]	93.5 (85.0; 102.1)	23.4 (19.9; 26.9)	40.1 (35.0; 45.1)	49.4 (44.3; 54.5)	57.0 (51.4; 62.7)
Saturated FA [1-7]	19.8 (18.1; 21.4)	5.6 (4.5; 6.7)	8.7 (7.1; 10.1)	10.7 (9.1; 12.2)	10.4 (9.3; 11.4)
Monounsaturated FA [1-10]	35.2 (31.7; 38.7)	8.6 (7.3; 9.9)	15.4 (13.1; 17.6)	19.4 (17.0; 21.7)	24.0 (21.1; 26.8)
Polyunsaturated FA [1-7, 9]	34.5 (31.3; 37.7)	8.4 (7.1; 9.7)	14.3 (12.7; 15.9)	17.7 (15.7; 19.7)	20.3 (18.2; 22.3)
Carbohydrates intake (g) [1-7, 9-10]	250.7 (226.4; 275.1)	86.0 (75.7; 96.3)	125.0 (109.9; 140.1)	143.0 (132.9; 153.2)	172.9 (158.0; 187.7)
Mono- & disaccharides (g) [1-4, 6-7]	99.0 (85.0; 113.1)	46.5 (39.4; 53.7)	60.9 (49.2; 72.6)	68.3 (59.7; 76.9)	76.9 (67.6; 86.1)
Polysaccharides (g) [1-10]	151.0 (139.2; 162.8)	36.9 (32.4; 41.5)	61.2 (54.0; 68.5)	75.0 (69.7; 80.3)	95.6 (89.0; 102.1)
Fibers (g) [1-3, 6-7, 9-10]	20.8 (19.3; 22.3)	11.1 (9.3; 12.9)	13.2 (11.7; 14.7)	14.4 (13.2; 15.7)	18.4 (16.8; 19.9)
Alcohol intake (g)[1]	4.9 (1.5; 8.2)	0.0 (0.0; 0.0)	6.4 (0.4; 12.4)	2.3 (0.7; 3.9)	1.6 (0.5; 2.8)
BMI (kg/m²) [1-10]	40.4 (39.4; 41.4)	36.6 (35.6; 37.5)	32.8 (31.9; 33.7)	29.7 (28.9; 30.6)	27.4 (26.5; 28.3)
EWL (%) [1-10]	0	21.1 (19.3; 23.0)	41.7 (39.3; 44.1)	58.0 (55.3; 60.7)	70.6 (67.9; 73.3)
FMI (kg/m²) [2-4, 8-10]	18.3 (17.1; 19.5)	-	12.1 (11.1; 13.1)	9.9 (8.9; 10.9)	7.9 (7.0; 8.7)
FFMI (kg/m²) [2-4, 8, 9]	22.3 (21.6; 23.0)	-	20.7 (20.1; 21.3)	19.8 (19.2; 20.4)	19.6 (19.0; 20.2)
Muscles (%) [2-4, 8-10]	14.1 (13.4; 14.9)	-	17.0 (16.1; 17.9)	17.7 (16.8; 18.7)	18.3 (17.4; 19.2)

[1] Significant difference (p<0.05) between pre-RYGB and 1M post-RYGB
[2] Significant difference (p<0.05) between pre-RYGB and 3M post-RYGB
[3] Significant difference (p<0.05) between pre-RYGB and 6M post-RYGB
[4] Significant difference (p<0.05) between pre-RYGB and 12M post-RYGB
[5] Significant difference (p<0.05) between 1M and 3M post-RYGB
[6] Significant difference (p<0.05) between 1M and 6M post-RYGB
[7] Significant difference (p<0.05) between 1M and 12M post-RYGB
[8] Significant difference (p<0.05) between 3M and 6M post-RYGB
[9] Significant difference (p<0.05) between 3M and 12M post-RYGB
[10] Significant difference (p<0.05) between 6M and 12M post-RYGB

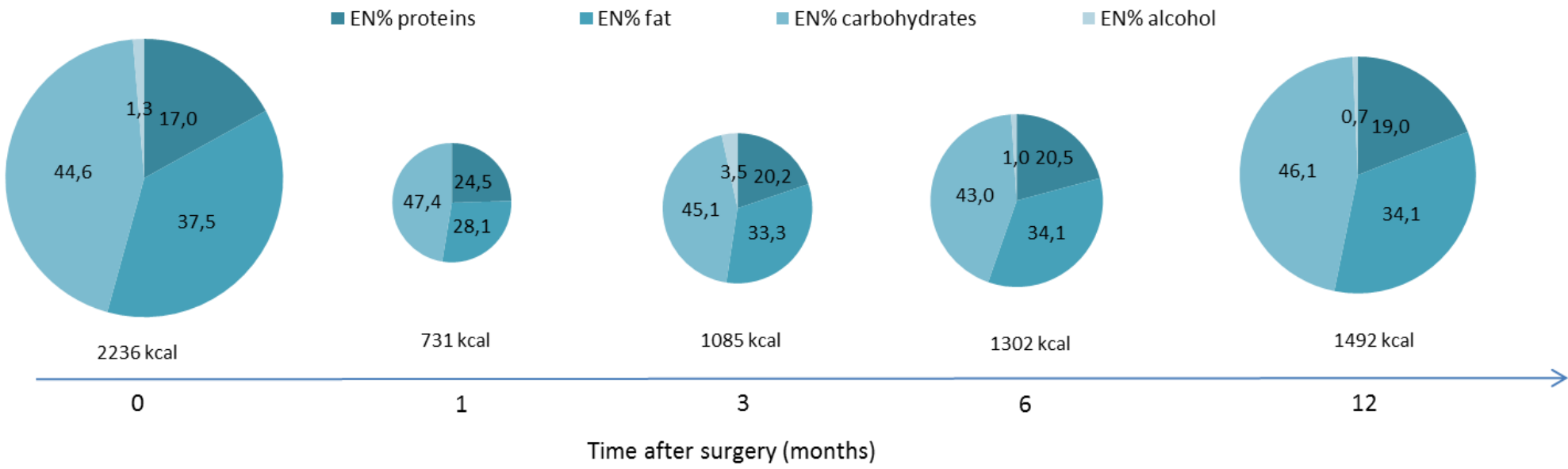

Figure 5: Percentage of macronutrients contributing to the energy intake

Despite the increase of protein intake with time after surgery, the percentage of patients with a daily intake below the recommended minimum of 60 g of proteins was still 35.2% and 14.8%, 6 and 12 months post-RYGB, respectively. This percentage was even 72.2% and 59.3% 1 and 3 months post-RYGB, respectively. An inadequate carbohydrate intake was observed 1, 3 and 6 months post-RYGB in 68.9%, 38.3% and 13.3% of the participants. One year post-RYGB, no participant had an inadequate total carbohydrate intake. Regarding fibers, most of the participants had an intake below the reference values of adequate intake of fibers. Before surgery, 83% of the participants had an inadequate fiber intake. This percentage further increased after surgery; 12 months post-RYGB, this percentage was 88%.

The ratio of the intake of polysaccharides versus mono- and disaccharides decreased significantly 1, 3 and 6 months post-RYGB compared to the preoperative situation (pre-RYGB ratio: 1.77). This ratio increased again with time after surgery to 1.45 one year post-RYGB. The percentage of participants with an inadequate intake of polyunsaturated fatty acids was enormously increased one month after surgery; from 3.8% before surgery to 86.7% one month-post-RYGB. This percentage decreased to 14.3% one year postoperative.

Furthermore, significant changes in body composition were observed. The FMI and FFMI were both significantly decreased 3 and 6 months post-RYGB and FMI further significantly decreased to one year post-RYGB. This is in contrast with the percentage of muscles, which significantly increased until one year post-RYGB, compared to baseline. A significantly, positive correlation between FFMI and the intake of proteins was observed ($p<0.01$). No statistically significant differences were observed in the measurement of the fat percentage by BIA and by DXA.

3.4 Discussion

The intake of energy and macronutrients was analyzed before and 1, 3, 6 and 12 months post-RYGB by collecting estimated dietary records. We observed that the energy and macronutrient intake was the highest before surgery and decreased enormously one month post-RYGB. Thereafter, the intake gradually increased again; even up to the baseline intake for some individuals. Furthermore, a significant decrease in FMI until one year and in FFMI until 6 months was observed and a significant correlation between protein intake and FFMI was identified.

The smaller caloric and macronutrient intake after surgery can be explained by the formation of a small gastric pouch during RYGB, which is associated with a reduced gastric capacity and subsequently restricted food intake [12]. Another contributing factor is probably the strict food regimen that is imposed to patients after bariatric surgery, evolving from liquid over semi-solid to solid food. This approach is in accordance with the clinical practice guidelines composed by the American Association of Clinical Endocrinologists (AACE), that stated that a liquid meal program needs to be started in the early postoperative care and that follow-up by the dietitian should be arranged for the initiation and progression of meals after bariatric surgery [36]. These different food phases also contribute to the lower intake.

After weight loss surgery, especially the intake of proteins is very important. Protein intake preserves fat free mass during the period of weight loss [36;44;100]. The significant correlation between protein intake and FFMI, observed in our study, confirms these results. However, one and 3 months post-RYGB, 72.2% and 59.3% of the participants, respectively, had a daily protein intake below 60 g. A comparable result, was shown in a study from Moizé et al. [44], in which 52.0% of the participants had a daily protein intake below 60 g, 4 months post-RYGB. In our study, one year post-RYGB, the percentage of patients below 60 g was decreased to 14.8%. The inadequate protein intake, especially during the first months after surgery, may be explained by red meat intolerances developed after RYGB [39]. These food intolerances decrease with time after surgery [108], probably explaining the

increase in protein intake with time after surgery, combined with the increased caloric intake over time. Furthermore, we need to take into account that the digestion and absorption of proteins might be reduced after RYGB; as the gastric acid and pepsinogen secretion is decreased and the inlet of secreted pepsin and other digestive enzymes is delayed postoperative [16;32]. Nevertheless, stimulating the intake of protein rich food such as meat, fish, poultry, vegetables and dairy products is very important after bariatric surgery to preserve lean body mass.

The percentage of patients with an inadequate intake of carbohydrates was smaller than for proteins. However, almost all patients had an inadequate fiber intake; this low intake is in line with previous studies [40;47]. The consumption of fruits, vegetables and whole grains needs to be supported to increase dietary fiber intake in this population group, this has also as advantage that it helps to prevent dumping syndrome [109].

A significant reduction in fat intake was also observed in the current study. The intake increased again with the time after surgery, although remaining below baseline values, which is similar to previous studies [17;20]. Individuals with a RYGB have lower preferences for food with a high fat content, which can explain the reduced fat intake after surgery [110]. The reduced fat intake was also associated with a higher percentage of patients with an inadequate intake of polyunsaturated fatty acids.

The alcohol intake is low at each postoperative time point, except 3 months post-RYGB. The low alcohol intake is comparable with previous studies regarding food intake after RYGB [40;111]. However, some studies have shown that patients after gastric bypass have an increased risk for alcohol dependence [112;113].

One year post-RYGB the energy intake from carbohydrates, proteins and fat was 46%, 19% and 34%, respectively. This distribution of macronutrients was comparable to the distribution shown in previous studies [43;114;115]. To prevent weight regain after RYGB a prescribed diet consisting of 45% carbohydrates, 35% proteins and 20% fat may be successful [36;116]. However, we need to take into

account that this diet advice is only based on one study, performed over a short period of time. According to these recommendations, the EN% from proteins in our study was still too low, while the intake from fat was too high, even though protein intake was already significantly increased and fat intake decreased compared to baseline. Thus, more comprehensive adjustments of dietary habits are required to maintain weight loss after RYGB.

Furthermore, we observed that with time after surgery the distribution of the EN% from the different macronutrients gradually evolved to the distribution before RYGB. These results may indicate recurrence of previous and unhealthy eating habits, comparable to the time before surgery. To investigate whether this trend continues with time after surgery, a longer follow-up of these patients could be interesting.

In this study, the prevalence of underreporting was high. A previous study has already shown that underreporting is more frequent in patients with a high BMI [117]. However, as we collected data within the same patient group, the collected data at the different time points were probably comparable regarding underreporting. The method used to determine underreporting has the disadvantage that BMR may be overestimated in obese patients, resulting in an overestimation of underreporting in this population group [107]. Furthermore, the use of the Goldberg cut-off is restricted to situations of weight stability, while our study population was losing weight, especially during the first months after surgery.

Obesity is associated with an abnormal or excessive fat mass accumulation and the objective of weight loss surgery is to reduce fat mass and maintain lean body mass [1]. We observed a significant decrease in BMI and FMI until one year post-RYGB, but there was also a significant decrease in FFMI the first six months post-RYGB; thereafter FFMI, remained stable. These results are consistent with previous studies [118-120]. Furthermore, we observed a significant correlation between protein intake and FFMI. In this study we did not collect data about physical activity, which has also an important influence on lean body mass. Combining data regarding protein intake and physical activity would

certainly be interesting. To estimate the body composition, we used a bio-impedance analysis. It is a measurement used in clinical practice that is portable, simple, inexpensive and noninvasive [121]. However, no information about regional changes in body composition can be measured with this method. Furthermore, errors with BIA measurements can occur as obese patients have subtle variations in hydration of soft tissues [122]. Some studies have already reported a good correlation between BIA measurements and DXA in overweight and obese individuals, but BIA has the tendency to overestimate FFM [123;124]. The good correlation is confirmed in this study as no significant differences were observed between the fat percentage measured by DXA and by BIA.

In this specific population group, dietary recommendations have not yet been developed for all nutrients. However, a lot of changes regarding nutrient absorption and nutritional requirements are associated with bariatric surgery. The use of dietary recommendations developed for the general population and comparison with reference values for the general population, is often not adequate in patients with bariatric history. As well recommendations based on expert opinion [125] need to be handled with care. Therefore, there is a high need for the development of dietary recommendations that are specific for this population group.

3.5 Conclusions

The energy and macronutrient intake was significantly decreased one month post-RYGB and gradually increased again with time after surgery. FMI significantly decreased until one year post-RYGB; FFMI significantly decreased until 6 months post-RYGB. A significant correlation was observed between protein intake and FFMI. Hence, it is important to stimulate RYGB-patients for an adequate protein intake.

CHAPTER 4: MICRONUTRIENT INTAKE, FROM DIET AND SUPPLEMENTS, AND THE ASSOCIATION WITH STATUS MARKERS IN RYGB PATIENTS BEFORE AND AFTER SURGERY

Manuscript in preparation

4 MICRONUTRIENT INTAKE, FROM DIET AND SUPPLEMENTS, AND THE ASSOCIATION WITH STATUS MARKERS IN RYGB PATIENTS BEFORE AND AFTER SURGERY

Background: Roux-en-Y gastric bypass (RYGB) is an effective treatment for morbid obesity. However, it is associated with an increased risk for the development of micronutrient deficiencies. The objective of this study was to assess the total intake (both dietary and from supplements) and status (including hepcidin) of iron, vitamin B_{12}, vitamin C, zinc and copper in patients before and after RYGB.

Methods: Patients who had planned RYGB surgery were included at University Hospitals Leuven, Belgium and were followed until 12 months post-RYGB. Patients completed an estimated dietary record of two non-consecutive days before and 1, 3, 6 and 12 months post-RYGB and supplement/drug use was questioned. Usual dietary intake of iron, vitamin B_{12}, zinc, copper and vitamin C was calculated and compared with Estimated Average Requirements (EAR). Furthermore, associations between total intake of these micronutrients and status markers were analyzed. Linear mixed models for repeated measures were used for analysis; statistical significance was set at $p<0.05$.

Results: Fifty-four patients (33♀, 21♂; mean age: 48.0 [95% CI 46.6; 49.3] years; mean preoperative BMI: 40.4 [95% CI 39.4; 41.4] kg/m²) were included. One month post-RYGB, the usual dietary intake of the studied micronutrients was significantly decreased compared to pre-RYGB, but gradually increased until 12 months after RYGB, remaining below baseline values. By including supplement intake of the micronutrients, 12 months post-RYGB values were higher than baseline, except for zinc. Hemoglobin, ferritin, vitamin B_{12} and C-reactive protein (CRP) serum concentrations were significantly decreased and transferrin saturation was significantly increased 12 months post-RYGB. Serum hepcidin concentration was significantly decreased 6 months post-RYGB.

Conclusions: Our data clearly suggest that medical nutritional therapy is essential following bariatric surgery as dietary intake of iron, vitamin B_{12}, vitamin C, copper and zinc is markedly decreased after RYGB. By including supplement intake of the micronutrients, there were still some patients with an inadequate intake of iron, copper and vitamin C one year post-RYGB, compared to the age- and

gender- specific EAR. The iron status was improved after RYGB by an increase in transferrin saturation and a decrease in serum hepcidin concentration. This trial was registered at clinicaltrials.gov as NCT01571180.

4.1 Introduction

Over the last decades, the prevalence of obesity has increased to epidemic proportions [1]. This is associated with an increased demand for bariatric surgery, which is considered the only efficient means, in combination with lifestyle interventions, to achieve major and sustainable weight reduction [98]. Nowadays, a Roux-en-Y Gastric bypass (RYGB) is the most commonly performed bariatric procedure. During a RYGB, a small gastric pouch is formed, which reduces the gastric capacity, and the duodenum and proximal jejunum are bypassed [12]. This results in a decreased food intake and absorption of food, which is reflected in an enormous decrease of energy and macronutrient intake during the first weeks after RYGB [17;28;40-42]. As a consequence of the low food intake, the intake of dietary micronutrients is low post-RYGB, increasing the risk for the development of nutritional deficiencies. Deficiencies in iron and vitamin B_{12} are common post-RYGB and can result in severe complications such as anemia [98;126].

Therefore, optimizing the diet by encouraging the intake of nutrient dense food is important. Additionally, the long term use of multivitamin/mineral supplements should be recommended to all patients after bariatric surgery to prevent the development of nutritional deficiencies. In some cases, additional supplementation may be required [36;100]. However, the intake from supplements is often ignored or not reported in research studies that have been performed about intake of micronutrients in patients with RYGB. Furthermore, the effect of the intake of micronutrients on status markers was mostly not investigated. In this study, we have included status markers that are standardly determined in clinical practice and we collected blood samples to determine hepcidin. Hepcidin has a central role in iron metabolism. It is a negative regulator of the iron metabolism by inhibiting intestinal absorption of iron, iron recycling by macrophages and mobilization of iron from hepatic stores [127]. To our knowledge, hepcidin concentrations have never been studied in patients with RYGB, only in patients who underwent a restrictive bariatric procedure [128]. Therefore, the objective of the current study was (1) to study both, the dietary and supplement intake of micronutrients (focusing on iron, vitamin B_{12}, vitamin C, zinc and copper) before RYGB and 1, 3, 6 and 12 months

after RYGB; and (2) to examine the association between the total micronutrient intake (both, from food and supplements/drugs) and the respective status markers.

4.2 Methods

4.2.1 Selection of patients

Obese patients who had planned RYGB surgery between April 2012 and January 2014 at the University Hospitals Leuven, Belgium, were asked to participate in the study. If patients consented to participate, they were included in the study. Patients with a bariatric surgery history and pregnant or lactating women were not included in the study. In all recruited patients, a laparoscopic RYGB with an alimentary limb of 120 cm and a small gastric pouch was performed by the same surgeon according to the same procedure. The study was approved by the Medical Ethics Committee of the University Hospitals Leuven (ML8063) and was registered at Clinicaltrials.gov (NCT01571180). All patients gave written informed consent.

4.2.2 Study design and data collection

The study was a prospective study, in which data were collected before surgery and 1, 3, 6 and 12 months after RYGB during the standard follow-up consultations at University Hospitals Leuven, Belgium.

Weight was measured to the nearest 0.1 kg, with the subjects wearing indoor clothing with empty pockets and without shoes. BMI (kg/m²) was calculated by dividing the weight (kg) by the square of height (m²). The percentage of excess weight loss was calculated using the formula:

$$\%\text{EWL} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight} - \text{ideal body weight}} \quad [102]$$

Patients were asked to keep an estimated dietary record during two non-consecutive days preceding each consultation. In this semi-structured dietary record, they noted all consumed foods and beverages using estimated amounts. The completeness and quality check of the dietary records was verified on consultation, which was always conducted by the same person (IG). An internal validation to identify underreporting was performed, as described in Chapter 3. After completion, the diaries

were processed into food quantities and codes by dietitians on the basis of a standard protocol, including a standard manual on food portions and household measures [103]. Based on this information, the actual intake of the micronutrients iron, vitamin B_{12}, vitamin C, copper and zinc was calculated. These micronutrients were chosen as iron and vitamin B_{12} deficiencies are common post-RYGB; vitamin C as the enhancer of iron absorption and copper and zinc as both minerals have a strong interaction with iron absorption and status [126;129]. For the determination of the nutrient intake, the Belgian Food Composition DataBase (FCDB) (NUBEL) has been used. If data were missing or incomplete in the Belgian FCDB table, the Dutch (NEVO), Finnish (Fineli), and American (USDA) databases were used in the respective order, taking into account whether products were fortified or not. Based on the actual intake, we estimated the usual dietary intake of the selected micronutrients, using the Multiple Source Method (MSM) [104], which is a statistical method for the calculation of usual intake, based on short-term measurement data. In this paper, dietary micronutrient intake is always shown as usual intake.

The usual dietary and total intake of micronutrients were compared with the Institute of Medicine (IOM) age- and gender- specific Estimated Average Requirements (EAR) [105] to determine the prevalence of inadequate intake of the studied micronutrients by using the EAR Cut-Off Method [130]. Furthermore, the total intake was compared with the estimated Recommended Dietary Allowances (RDA) after RYGB, compiled by Valentino et al. [125]. In Figures, EAR, RDA and the estimated RDA after RYGB are shown.

At each time point, also the use of supplements and medical drugs was questioned. Medical drugs containing nutrients were taken into account to calculate the total daily supplement intake. Regarding the supplemental intake, the following assumptions were used: if a preparation had to be taken for example only once a week, the dosage was divided by 7 to calculate the daily intake; when patients indicated they had a low adherence (=participant's perception; if the participant indicated that he/she missed more than half of the doses), the intake of micronutrients from the appropriate

supplement/drug was not taken into account for the calculation of the total intake. The intake of micronutrients by another route than oral administration was not taken into account, neither from herbal preparations. The total daily oral intake of micronutrients could be calculated by counting the usual dietary and supplement intake.

Status markers (hemoglobin, ferritin, transferrin saturation, vitamin B_{12} and zinc) were collected before and 6 and 12 months after surgery. These concentrations were determined by the Clinical Laboratory of the University Hospitals Leuven. Anemia was defined as hemoglobin < 14 g/dL for men and < 12 g/dL for women. Vitamin B_{12} deficiency was defined as a serum concentration of vitamin B_{12} < 200 ng/L; iron deficiency as serum ferritin < 30 µg/L and/or transferrin saturation (TSAT) < 20%; zinc deficiency as a serum concentration of zinc < 60 µg/dL. Inflammation markers C-reactive protein (CRP) and hepcidin were measured. Hepcidin concentrations before and 3, 6 and 12 months after RYGB were determined using the hepcidin-25 (human) Enzyme-Linked Immunosorbent Assay (ELISA) kit from Peninsula Laboratories International, Inc.

4.2.3 Statistical analyses

Linear models for repeated measurement were used to analyze the evolution of dietary intake over time and to identify associations between total micronutrient intake (including dietary and supplement intake) and status markers. Time was modeled categorically, and an unstructured residual covariance matrix was modeled to account for intra-subject correlation. The Sidak multiple test correction was used. Data are shown as estimated mean and 95% confidence interval (95% CI), except otherwise mentioned. Statistical significance was set at $p<0.05$. The data were analyzed with SPSS Statistics 22.

4.3 Results

There were 54 participants (33♀, 21♂) included in this study with a mean age of 48.0 (95% CI 46.6; 49.3) years. The mean BMI before surgery was 40.4 (95% CI 39.4; 41.4) kg/m², which decreased to 27.4 (95% CI 26.5; 28.3) kg/m² 12 months post-RYGB, which corresponds to 70.6% (95% CI 66.5; 74.7) of excess weight loss.

The usual dietary intake of energy, iron and vitamin B_{12} was enormously decreased one month post-RYGB, but gradually increased until 12 months post-RYGB, as shown in Table 14. A similar trend was observed for copper and vitamin C, but with a smaller increase of intake after one month post-RYGB. The dietary intake of zinc was the lowest 3 months post-RYGB, and then started to increase again. The mean usual dietary intake of all the micronutrients remained below baseline values at each time point. Table 14 also shows the evolution of the total intake of micronutrients (sum of dietary and supplement intake) over time.

Figure 6 shows the total intake of the micronutrients (sum of dietary and supplement intake) with indication of the lower limit of EAR, RDA and RDA after RYGB. When comparing the total intake of each micronutrient at the different time points, a significant reduction one month post-RYGB was observed for all the studied micronutrients. Afterwards, the total micronutrient intake gradually increased until 12 months post-RYGB to values higher than baseline values, except for total intake of zinc. For all the studied micronutrients, there was a significant difference in total intake between 1 and 12 months post-RYGB.

Table 14: Estimated means (95% CI) of usual dietary intake and total intake of micronutrients before, and 1, 3, 6 and 12 months post-RYGB

Micronutrient	Before RYGB	1 month post-RYGB	3 months post-RYGB	6 months post-RYGB	12 months post-RYGB
Energy (kcal) [1-10]	2235.7 (2053.4; 2418.0)	731.3 (647.0; 815.6)	1084.8 (987.1; 1182.6)	1301.7 (1204.6; 1398.9)	1491.9 (1387.5; 1596.4)
Dietary iron (mg) [1-3, 7, 9]	12.9 (12.5;13.3)	6.4 (4.3;8.5)	7.2 (6.4; 8.1)	8.1 (7.1; 9.0)	10.5 (8.9;12.2)
Total iron (mg) [1, 4, 7, 9, 10]	12.9 (12.5;13.3)	6.4 (4.3;8.5)	11.2 (4.9; 17.5)	22.6 (11.5; 33.6)	42.3 (28.9; 55.6)
Dietary vitamin B_{12} (µg) [1-7, 9]	5.9 (5.4; 6.3)	2.61 (2.1; 3.0)	3.9 (3.8; 4.1)	4.3 (3.8; 4.8)	4.7 (4.3; 5.1)
Total vitamin B_{12} (µg) [4, 7, 9]	15.7 (-3.9; 35.3)	22.2 (-5.9; 50.3)	22.0 (-2.5; 46.5)	38.5 (6.2; 70.8)	98.6 (50.2; 146.9)
Dietary zinc (mg) [1-4, 9, 10]	56.9 (32.1; 81.8)	11.5 (7.6; 15.3)	10.6 (8.5; 12.7)	11.3 (8.6; 14.1)	16.6 (15.1; 18.2)
Total zinc (mg) [1-3, 7, 9, 10]	58.7 (32.5; 84.9)	12.1 (8.0; 16.2)	12.1 (9.7; 14.4)	15.2 (11.3; 19.1)	26.0 (21.7; 30.2)
Dietary copper (mg) [1, 5]	2.7 (2.2; 3.2)	1.6 (1.3; 2.0)	2.5 (1.9; 3.0)	1.9 (1.4; 2.3)	2.0 (1.7; 2.4)
Total copper (mg) [1, 5, 7]	2.8 (2.3; 3.3)	1.7 (1.3; 2.1)	2.6 (2.0; 3.1)	2.2 (1.6; 2.7)	3.0 (2.6; 3.5)
Dietary vitamin C (mg) [1-4]	114.3 (99.9; 128.7)	64.6 (50.4; 78.9)	81.0 (71.7; 90.4)	66.1 (56.8; 75.5)	72.1 (61.1; 83.0)
Total vitamin C (mg) [1, 7, 10]	118.4 (103.0; 133.9)	76.6 (46.2; 106.9)	100.2 (75.3; 125.2)	90.1 (71.7; 108.4)	141.2 (117.6; 164.7)

[1] Significant difference (p<0.05) between pre-RYGB and 1M post-RYGB
[2] Significant difference (p<0.05) between pre-RYGB and 3M post-RYGB
[3] Significant difference (p<0.05) between pre-RYGB and 6M post-RYGB
[4] Significant difference (p<0.05) between pre-RYGB and 12M post-RYGB
[5] Significant difference (p<0.05) between 1M and 3M post-RYGB
[6] Significant difference (p<0.05) between 1M and 6M post-RYGB
[7] Significant difference (p<0.05) between 1M and 12M post-RYGB
[8] Significant difference (p<0.05) between 3M and 6M post-RYGB
[9] Significant difference (p<0.05) between 3M and 12M post-RYGB
[10] Significant difference (p<0.05) between 6M and 12M post-RYGB

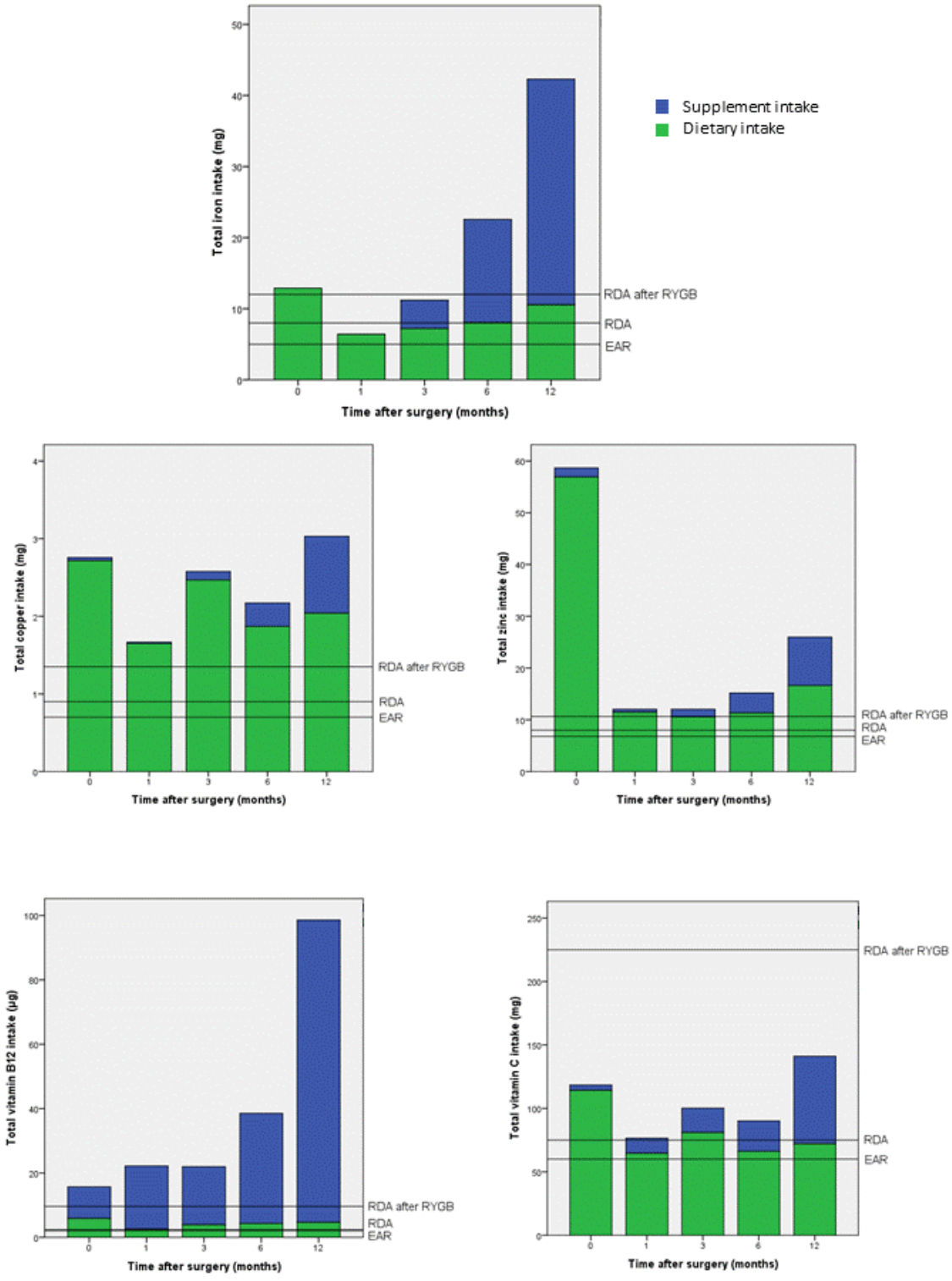

Figure 6: Mean iron, zinc, copper, vitamin B_{12} and vitamin C intake from food and supplements/drugs with the lower limit of EAR, RDA and RDA after RYGB

By comparing the dietary and total intake of the studied micronutrients with the corresponding age- and gender- specific EAR at the different time points, the percentage of patients with an intake below EAR was the highest one month post-RYGB, as shown in Table 15. If the total intake of the different micronutrients was compared to the age- and gender- specific RDA after RYGB composed by Valentino et al. [125], more than 80% of the participants had a lower total iron and vitamin B_{12} intake the first six months post-RYGB, and 12 months post-RYGB this percentage was still 48% and 69%, respectively. Regarding total vitamin C, more than 90% had a vitamin C intake below RDA after RYGB at each postoperative time point. The percentages of participants with a total zinc and copper intake below RDA after RYGB were slightly lower. The first six months after RYGB, more than 50% had a zinc intake below RDA after RYGB and more than 35% had a copper intake below RDA after RYGB. These percentages decreased to 12% for zinc and 19% for copper.

Table 15: Percentage of patients with a dietary and total intake below the corresponding age- and gender-specific EAR

Micronutrient	Before RYGB (%)	1 month post-RYGB (%)	3 months post-RYGB (%)	6 months post-RYGB (%)	12 months post-RYGB (%)
Dietary iron	0.0	60.0	44.7	28.9	14.3
Total iron	0.0	60.0	38.3	22.2	7.1
Dietary vitamin B_{12}	0.0	40.0	0.0	4.4	0.0
Total vitamin B_{12}	0.0	37.8	0.0	4.4	0.0
Dietary zinc	1.9	46.7	40.4	42.2	2.4
Total zinc	1.9	44.4	31.9	35.6	0.0
Dietary copper	0.0	28.9	10.6	13.3	4.8
Total copper	0.0	28.9	10.6	11.1	2.38
Dietary vitamin C	15.1	66.7	34.0	55.6	54.8
Total vitamin C	13.2	66.7	29.8	42.2	21.4

An overview of status markers, collected before and 6 and 12 months post-RYGB, is shown in Table 16. The plasma concentrations of hemoglobin and ferritin decreased significantly over the first year post-RYGB. Before surgery, 9.2% suffered already from anemia, and this number increased with time after surgery to 22% and 28.3% 6 and 12 months post-RYGB. Iron deficiency was found in 49.1% of the patients before surgery and 6 and 12 months after surgery, this percentage was 38.0% and 34.0%, respectively.

Twelve months post-RYGB, the mean plasma concentration of transferrin saturation was significantly increased compared to baseline. The mean hepcidin concentration was significantly decreased 6 months post-RYGB, compared to baseline. However, 3 months post-RYGB there was a small increase in mean hepcidin concentration compared to baseline; from 41.9 (95% CI 30.7; 53.1) before surgery to 43.2 (95% CI 33.7; 52.7) 3 months post-RYGB. By comparing vitamin B_{12} plasma concentration levels at 6 and 12 months post-RYGB with baseline values, a significant decrease was observed. This is associated with an increase in patients with a vitamin B_{12} deficiency, from 3.8% before surgery to 24.0% and 23.4% 6 and 12 months post-RYGB, respectively. Furthermore, the mean CRP plasma concentration was significantly decreased 6 months post-RYGB, and further decreased until 12 months post-RYGB. Regarding plasma concentrations of zinc, no significant differences were observed. In Figure 7, the evolution of hepcidin and TSAT is shown over time after RYGB.

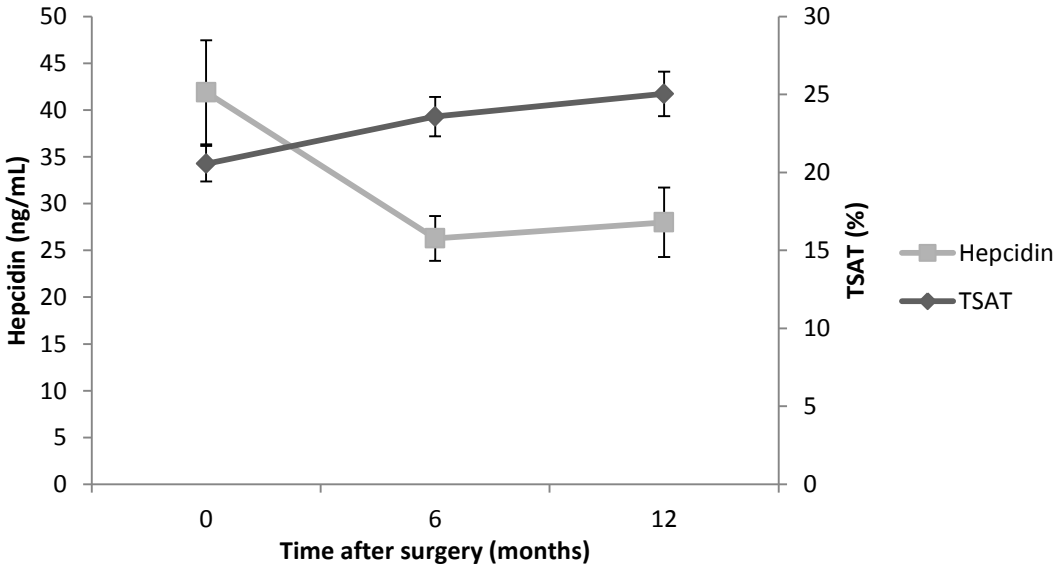

Figure 7: Evolution of hepcidin and TSAT over time, shown as mean±SEM

Table 16: Overview of estimated means (95% CI) of the total micronutrient intake and the clinical parameters before and 6 and 12 months post-RYGB

Parameter	Reference range	Before RYGB	6 months post-RYGB	12 months post-RYGB
BMI (kg/m²)		40.5 (39.5; 41.5)	29.8 (28.9; 30.7)*	27.4 (26.5; 28.3)*
%EWL		0.0 (0.0; 0.0)	58.0 (55.3; 60.7)*	70.6 (67.9; 73.3)*
CRP (mg/L)	≤ 5	6.8 (4.8; 8.9)	2.3 (1.4; 3.2)*	1.0 (0.5; 1.6)*
Total iron intake (mg)		12.9 (12.4; 13.4)	22.6 (11.5; 33.6)	42.3 (28.9; 55.6)*
Hemoglobin (g/dL)	♂ 14-18; ♀ 12-16	14.2 (13.8; 14.5)	13.5 (13.2; 13.8)*	13.4 (13.1; 13.7)*
Transferrin saturation (%)	16-45	20.6 (18.3; 22.8)	23.6 (21.0; 26.1)	25.0 (22.2; 27.9)*
Ferritin (µg/L)	♂ 30-400; ♀ 13-150	174.8 (131.0; 218.7)	121.6 (92.8; 150.4)*	90.3 (67.8; 112.8)*
Hepcidin		41.9 (30.7; 53.1)	26.3 (21.5; 31.1)*	28.0 (20.5; 35.5)
Total vitamin B_{12} intake (µg)		15.7 (-3.9; 35.3)	38.5 (6.2; 70.8)	98.6 (50.2; 146.9)*
Vitamin B_{12} (ng/L)	191-663	385.9 (338.5; 433;2)	275.9 (243.7; 308.1)*	307.3 (269.3; 345.4)*
Hemoglobin (g/dL)	♂ 14-18; ♀ 12-16	14.2 (13.8; 14.5)	13.5 (13.2; 13.8)*	13.4 (13.1; 13.7)*
Total zinc intake (mg)		58.7 (32.5; 84.9)	15.2 (11.3; 19.1)*	26.0 (21.7; 30.2)
Zinc (µg/dL)	71-109	84.4 (80.0; 88.1)	81.8 (76.8; 86.9)	83.8 (80.2; 87.5)

*p<0.05 compared to baseline

By analyzing potential correlations between the total micronutrient intake and clinical parameters, a significant correlation between vitamin B_{12} intake and vitamin B_{12} plasma concentration (slope=0.24 [95% CI 0.14; 0.34]; p<0.01) was observed. No other statistically significant correlations were identified.

4.4 Discussion

In this prospective study, we have collected extensive, reliable data about dietary and supplement intake and have determined concentrations of status markers. We confirmed previous findings regarding dietary intake, namely that the dietary intake of iron, vitamin B_{12}, vitamin C and copper is enormously decreased one month post-RYGB, but gradually increases until 12 months post-RYGB, although remaining below baseline values. The dietary intake of zinc was the lowest 3 months post-RYGB, and then started to increase again. When supplement use was taken into account, a similar pattern of micronutrient intake was observed (except for vitamin B_{12}), with the difference that the intake 12 months post-RYGB was higher than before surgery.

The observed dietary intake pattern of iron and vitamin B_{12} is in line with previous studies [40;41;46;48]. This pattern might be associated with reduced red meat consumption. Food preferences change after RYGB and some patients develop food intolerances, especially the first months after surgery. One of the most common food intolerances is the one for red meat, a major source of iron and vitamin B_{12} [39].

In our study, an inadequate dietary iron intake was observed in 60 and 45% of the participants 1 and 3 months post-RYGB, respectively. This number decreased to 29 and 14%, 6 and 12 months post-RYGB, respectively. In the current study the prevalence of inadequate iron intake is, compared to other studies, lower [40;42]. A potential explanation could be the fact that we transformed actual intake to the usual dietary intake, as recommended by the Institute of Medicine (IOM) to assess the prevalence of inadequacy [130]. We also compared the dietary intake with the EAR instead of the RDA as some other studies do, as is recommended by IOM.

Hemoglobin concentration was significantly decreased as soon as 6 months post-RYGB, which reflects the high prevalence of anemia post-RYGB. This is in line with the results of Ruz et al. (n=67) who have shown an increase of anemic patients from 1.5% before RYGB to 38.8% 18 months after surgery [24]. The significant decrease of CRP and ferritin postoperatively, observed in our study confirms the improvement of the obesity-associated low-grade systemic inflammation [131].

We showed that the iron status was improved and the percentage of patients with an iron deficiency was decreased post-RYGB. This could be explained by the intake of iron supplements and the decreased obesity-associated inflammation. The transferrin saturation was significantly increased 12 months post-RYGB. Hepcidin concentration was significantly decreased 6 months post-RYGB, which also reflects the improvement of the obesity-associated inflammation. The decreased hepcidin concentration is potentially related to the increase of transferrin saturation and is a possible indication for enhanced iron absorption after RYGB and subsequently the improved iron profile. It needs to be mentioned that the mean hepcidin concentration was not yet decreased 3 months post-RYGB. This can possibly be explained by the fact that the inflammation was not yet improved so soon after surgery and supplement use was limited at that time point. According to our knowledge, this is the first study that also included the measurement of hepcidin in RYGB-patients. Another study has investigated hepcidin concentrations after restrictive bariatric surgery and they observed similar results [128]. Hepcidin levels modulation could potentially be considered as an adjuvant therapy instead of high doses of iron supplementation.

The percentage of patients with an inadequate dietary intake of vitamin B_{12} in our study was smaller than for iron and even null before and 3 and 12 months post-RYGB, which is in line with previous studies [40;43;46;48]. Despite the small percentage of patients with an inadequate intake of vitamin B_{12}, a lot of patients suffered from vitamin B_{12} deficiency. The development of vitamin B_{12} deficiency after RYGB is probably due to absorption problems and not to an insufficient intake of vitamin B_{12}. This can

be explained by the reduced gastric acid and intrinsic factor secretion after RYGB, essential for the release of vitamin B_{12} from proteins and the absorption of vitamin B_{12}, respectively [31].

More than 40% of the participants had a dietary intake of zinc below EAR 1, 3 and 6 months post-RYGB and the dietary zinc intake decreased until 3 months post-RYGB; afterwards there was a small increase until 12 months post-RYGB, which is in line with previous studies [27;40;46;51]. The remarkable high intake of zinc, especially one month post-RYGB, is related to the high intake of soft drinks, which contains a high amount of zinc, of some participants. We need to take into account a large inter-individual variation of dietary zinc intake, which was also observed in a study by Cominetti et al. [51]. The dietary copper intake was also significantly decreased one month post-RYGB and underwent a small increase afterwards. However, the mean dietary intake was higher than the RDA after RYGB at all time points.

More than 50% of the participants had a dietary vitamin C intake below EAR at 1, 6 and 12 months post-RYGB; this low dietary vitamin C intake post-RYGB has also been observed by others [40;48;50]. This could be explained by the fact that patients after RYGB often do not change or resume their dietary habits from before, i.e. still consume low quality food with a small intake of fruit and vegetables. This inadequate intake of vitamin C could result in a decrease in iron absorption after RYGB, as vitamin C enhances the absorption of iron [132].

Regarding the intake of micronutrients from supplements, there was an increase with time after surgery since in the hospital, in which the study was performed, multivitamin/mineral supplements are not started immediately after surgery, but are mostly recommended as soon as six months after bariatric surgery. By adding supplement intake of micronutrients to the dietary intake, we still found patients with an inadequate intake of the different micronutrients (compared to the EAR) at the different time points post-RYGB, except for vitamin B_{12} at 3 and 12 months postoperatively and for zinc 12 months after surgery. By comparing the total micronutrient intake with the RDA after RYGB, published by Valentino et al. [125], a high percentage of participants had an iron, vitamin B_{12} and

vitamin C intake below these values. However, one should take into account that these RDA values are based on general findings of deficiencies in different studies, and should be used with caution. Therefore, the development of Food-Based Dietary Guidelines for this specific population would be a very interesting route to follow in order to provide patient specific nutritional guidance.

A current practice to compensate the low dietary intake of several micronutrients is the use of supplements. However, there is currently a lack of evidence on the upper level of supplementation. A risk of uncontrolled supplementation of high doses of micronutrients is an overload of micronutrients, which has been observed in a recent study [133]. In this study, high dose supplement was compared to a standard multivitamin/mineral supplement. Regarding iron metabolism, no significant differences were observed between both treatment groups. However, a hypervitaminosis for vitamin B_{12} was observed in 16 of 148 RYGB-patients taking a daily multivitamin supplement [133]. These findings raise the question if the long-term use of high-dose supplements will not lead to an overload. Therefore, measurement of non-transferrin bound iron could be interesting as it has a key role in iron toxicity. Circulating non-transferrin bound iron is present in the bloodstream independently of transferrin. It is known that circulating non-transferrin bound iron can appear independently of the presence of available binding sites on transferrin on the condition that rate of iron influx into plasma exceeds the rate of iron acquisition by transferrin [134;135].

Furthermore, anatomical and physiological changes after RYGB need to be taken into account when choosing a supplement, as these can result in altered bioavailability of micronutrients. Therefore, changing the type of the different micronutrients such as the salt form or amino chelate, and the formulation of supplements can contribute to an improved absorption. For instance, calcium citrate seems to be better absorbed after RYGB than calcium carbonate; in a previous study the mean serum concentration and peak plasma concentration of calcium were significantly higher after oral administration of calcium citrate compared to calcium carbonate [29]. Furthermore, Sakhaee et al.

have shown that the bioavailability of calcium from an effervescent tablet was higher than from a regular tablet formulation [136].

Moreover, changes in gut microbiota after RYGB and intestinal adaptation can play a role in nutrient absorption. The latter has already been shown for carbohydrate absorption with an up-regulation of intestinal glucose transporters after RYGB surgery [137;138]. However, further research regarding nutrient absorption is needed.

Nevertheless, supplement use remains very important for patients who consume less nutrient dense food in order to obtain adequate intake of the different micronutrients. Health care professionals have an important double role, first to encourage these patients to adapt a healthy dietary pattern and secondly to stimulate the adherence of supplement use.

The current study has some strengths and limitations. First of all, an important strength of our study is that we collected data about dietary and supplement intake and also determined status markers. We have chosen to use 2-days dietary records to collect data about food intake as it is assumed to give a realistic and reliable estimation. When patients need to fill in a dietary record during more days, the adherence to the recording reduces [139]. Previous studies have as limitation that the analyses were based on actual dietary intake. Therefore, we transformed actual to usual dietary intake, which has the advantage that the analyses are based on a population distribution, calculated on the collected individual data. Furthermore, the added value of this study is that we have taken into account supplement use, which is important as a large proportion of micronutrient intake comes from supplement use in this patient group and this intake is often ignored in previous studies. However, we need to take into account that the quantity of micronutrients in supplements may vary as a margin for variation of the quantity mentioned on the label of supplements, is allowed. According to this principle it is not possible to calculate the exact intake of micronutrients from supplements. Both, the transformation to usual intake and including the supplemental intake of micronutrients, are important advantages of this study and allow our data to be used for the

development of micronutrient recommendations, according to the EURRECA principle [140]. Furthermore, we included status markers. Especially the measurement of hepcidin has an important added value. Finally, the current results only reflect 12 months after RYGB surgery; a longer follow-up could be useful in order to determine whether the total (both, from food and supplements/drugs) intake of micronutrients continues to increase with time after surgery.

This study indicates that a specific medical nutrition therapy is essential after bariatric surgery to optimize micronutrient status. The specific medical nutrition therapy should include (1) specific food-based dietary guidelines which focus on nutrient dense food and take into account the altered bio-availability aspects of these specific patient population and (2) specific targeted multimineral/vitamin supplements and aims to optimize the micronutrient status to prevent the development of micronutrient deficiencies avoiding any form of over-supplementation.

4.5 Conclusions

The dietary intake of iron, vitamin B_{12}, vitamin C, copper and zinc is markedly decreased after RYGB. By including supplement intake of micronutrients, there were still some patients with an inadequate intake of iron, copper and vitamin C one year post-RYGB, compared to the age- and gender- specific EAR. Our data clearly suggest that medical nutrition therapy is essential following bariatric surgery. Moreover, the iron status was improved after RYGB by an increase in transferrin saturation and a decrease in serum hepcidin concentration.

PART III: INFLUENCE OF RYGB ON IRON STATUS AND ITS ABSORPTION

CHAPTER 5: IRON DEFICIENCY AFTER ROUX-EN-Y GASTRIC BYPASS: INSUFFICIENT IRON ABSORPTION FROM ORAL IRON SUPPLEMENTS

This chapter is based on:

Gesquiere, I., Lannoo, M., Augustijns, P., Matthys, C., Van der Schueren, B., Foulon, V. (2014) Iron deficiency after Roux-en-Y Gastric Bypass: insufficient iron absorption from oral iron supplements

Obesity Surgery, 24(1), 56-61.

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5 IRON DEFICIENCY AFTER ROUX-EN-Y GASTRIC BYPASS: INSUFFICIENT IRON ABSORPTION FROM ORAL IRON SUPPLEMENTS

Background: Roux-en-Y gastric bypass (RYGB) may reduce the absorption of iron, but the extent to which this absorption is impeded is largely unknown. First, we determined the prevalence of iron deficiency following RYGB and explored the risk factors for its development. Second, we examined to what extent oral iron supplements are absorbed after RYGB.

Methods: Monocentric retrospective study in 164 patients (123 females, 41 males; mean age 43 years) who underwent RYGB between January 2006 and November 2010 was done. Pre-and postoperative data on gender, age, BMI, serum levels of iron, ferritin, hemoglobin, vitamin B_{12}, 25-hydroxy vitamin D, and use of proton pump inhibitors and H_2 antagonists were collected. Generalized linear mixed models were used for the analysis of the data. In 23 patients who developed iron deficiency after surgery, an oral challenge test with 100 mg $FeSO_4.7H_2O$ was performed.

Results Following RYGB, 52 (42.3%) female patients and 9 male (22.0%) patients developed iron deficiency (serum ferritin concentration ≤ 20 µg/L). The prevalence of iron deficiency was significantly higher in females than males (p=0.02). Young age (p=0.01), poor preoperative iron status (p<0.01), vitamin B_{12} deficiency (p<0.01) and increasing time after surgery (p<0.01) were also associated with iron deficiency. In the oral iron challenge test, only one patient out of 23 showed sufficient iron absorption.

Conclusions: Iron deficiency is extremely frequent after RYGB and is linked with different risk factors. Iron supplementation seems essential, but the effect of oral tablets may be limited as absorption of oral iron supplements is insufficient post-RYGB.

5.1 Introduction

Over the last decades, there has been an increase in the prevalence of obesity, which is now considered a global epidemic [1]. As a consequence, the number of Roux-en-Y gastric bypasses (RYGB) performed has also surged, as it is currently the only intervention in combination with lifestyle modifications which achieves major and sustainable weight reduction [141]. However, RYGB may be associated with nutritional deficiencies [11]. Deficiencies in iron, vitamin B_{12}, folic acid, vitamin D, and calcium are the most common manifestations of nutritional problems post-RYGB, some of which can result in severe complications such as anemia [98;126;142]. Iron deficiency has been reported in 20 to 49% of patients following RYGB [23;143]. This deficiency can be explained by three factors: (1) diminished gastric acid secretion, which is necessary for the absorption of iron; (2) reduced intestinal absorption surface, particularly the excision of the duodenum, the main absorption site of iron; and (3) low tolerance to red meat, a major source of iron [11;24;144;145].

Therefore, patients after RYGB are routinely recommended to take prophylactic oral iron supplements [25]. Unfortunately, even upon correct administration of iron tablets, oral replacement therapy (usually with up to 300 mg of elemental iron per day in three or four iron tablets [146]) often fails to correct the deficiency in a large proportion of patients.

The objectives of the current study were twofold: (1) to determine the prevalence of iron deficiency post-RYGB and explore potential risk factors by evaluating the association between ferritin status and gender, BMI, age, time since surgery, medication use and other vitamin deficiencies and (2) to assess absorption of iron after a standardized oral challenge test in iron-deficient patients after surgery.

5.2 Methods

5.2.1 Retrospective analysis of patient records

A first part of the study (performed in March 2011) consisted of a retrospective analysis of records of patients who had undergone a RYGB between January 2006 and November 2010 in the University

Hospitals Leuven, Belgium. In all patients a laparoscopic gastric bypass with an alimentary limb of 120cm and a small gastric pouch was performed.

For each patient, data were collected from the preoperative consultation and, dependent on the time of the surgery, from the consultations at 6, 12, 24, 36, 48 and 60 months postoperative, if available. There were no limits regarding gender or age. Patients who had undergone a previous bariatric surgery were excluded from the study.

The following parameters were included into the analysis: gender, age, BMI, serum levels of iron, ferritin, hemoglobin, vitamin B_{12}, and 25-hydroxy vitamin D. Furthermore, we included the use of proton pump inhibitors, H_2 antagonists, or antacids. If the ferritin level at some point during follow-up (ranging between 6 and 60 months) was ≤ 20 µg/L, the patient was considered to suffer from iron deficiency. Anemia was defined as hemoglobin ≤ 12 g/dL in women and ≤ 14 g/dL in men, and vitamin B_{12} deficiency was defined as serum concentration of vitamin B_{12} ≤ 180 ng/mL.

The study was approved by the Medical Ethics Committee of the University Hospitals Leuven (ML7750).

5.2.2 Oral challenge test

Twenty-three patients from the retrospective study who had developed iron deficiency (ferritin level < 20 µg/L) after RYGB, received an oral challenge test with 100 mg $FeSO_4.7H_2O$ [147]. These oral iron absorption tests were conducted as a standard procedure in clinical practice before initiating intravenous iron. The patients did not use iron supplements and did not receive a transfusion of red blood cells during the 3 weeks preceding the oral challenge test. One week before the test, the use of proton pump inhibitors, H_2 antagonists, antacids, and vitamin supplements was discontinued.

Patients remained fasted during the entire test. In the fasted state (t=0), a blood sample was collected to determine serum iron, ferritin, transferrin and transferrin saturation. Subsequently, a capsule containing 100 mg $FeSO_4.7H_2O$ was administered with a glass of water. After the ingestion of iron, blood samples were taken at t=1, 2 and 3 h. The estimation of the extent of the intestinal

absorption of iron was determined as the difference between the highest serum iron concentration obtained 1, 2 or 3 h following the ingestion of iron, and the serum iron concentration at *t*=0. An increase of 80 µg/dL was considered to be representative for sufficient iron absorption [147].

5.2.3 Statistical analysis

Variables are summarized by means and standard deviations (SD). The data contain longitudinal measurements on the patients with assessments made at preoperative consultation and at 6, 12, 24, 36, 48 and 60 months postoperative. Nevertheless, not for all patients data are available at all time points due to missing information in files, missed consultations or closing of data collection. Summary statistics are based on available data and restricted to records with available ferritin measurement. Generalized linear mixed models were used for the analysis of the data, with iron deficiency as binary response variable, and a random intercept accounting for correlation between repeated measurements coming from the same patient. Inference is likelihood-based and valid if drop-out is missing at random, in the sense that missingness may depend on previous outcomes or covariates but no further on unobserved outcomes [148]. Covariate effects are presented as odds ratios (OR) and their 95% confidence intervals (CI). Effects were considered to be statistically significant when $p<0.05$. The analyses were performed using the NLMIXED procedure in SAS, version 9.2 of the SAS System for Windows.

5.3 Results

5.3.1 Prevalence of iron deficiency post-RYGB and predictive parameters

Data from 164 patients were collected: 123 females (75.0%) and 41 males (25.0%), mean age of 43 years (SD 10 years), and mean BMI of 41.8 kg/m² (SD 4.9 kg/m²). An overview of the clinical measurements at the different moments is shown in Table 17.

Table 17: Summary statistics, shown as mean (standard deviation)

	Before surgery (***n*=130**)	**6 months after RYGB** (***n*=107**)	**1 year after RYGB** (***n*=90**)	**2 years after RYGB** (***n*=55**)	**3 years after RYGB** (***n*=44**)	**4 years after RYGB** (***n*=27**)	**5 years after RYGB** (***n*=19**)
Gender	99♀, 31 ♂	86♀, 21 ♂	67♀, 23♂	39♀, 16♂	33♀, 11♂	18♀, 9♂	15♀, 4 ♂
Age (years)	43.1 (12.0)	42.2 (11.4)	42.1 (12.0)	44.3 (12.0)	42.4 (12.8)	43.6 (13.6)	41.2 (11.7)
Ferritin (µg/L)	101.7 (115.7)	81.3 (78.9)	76.9 (84.0)	66.3 (70.8)	35.6 (54.1)	56.2 (83.9)	38.4 (46.5)
Hemoglobin (g/dL)	14.0 (1.5)	13.4 (1.8)	13.3 (1.3)	13.3 (1.5)	13.5 (1.5)	13.5 (1.0)	13.5 (1.3)
Weight (kg)	117.6 (17.0)	86.5 (12.5)	80.2 (14.5)	84.8 (18.6)	90.2 (16.0)	98.7 (5.5)	75.4 (5.2)
BMI (kg/m²)	42.0 (4.9)	30.9 (3.7)	28.6 (4.1)	29.5 (5.2)	31.6 (4.6)	36.5 (1.9)	26.7 (1.8)
Vitamin B_{12} (ng/mL)	301.0 (199.5)	270.9 (189.3)	264.5 (148.0)	266.8 (166.4)	317.2 (268.4)	261.5 (146.0)	361.4 (287.5)
Vitamin D (µg/L)	11.2 (13.3)	21.0 (13.0)	20.2 (12.9)	17.8 (11.1)	19.7 (11.4)	21.4 (13.9)	22.3 (17.5)

After surgery, 61 patients (37.2%) developed iron deficiency at some point during follow-up after surgery (follow-up duration varying between 6 months and 5 years): 52 (42.3%) female patients and 9 male (22.0%) patients. The probability for males to develop iron deficiency post-RYGB was significantly lower compared to females [OR=0.18 (95% CI 0.04; 0.73); p=0.017]. Low iron status resulted in anemia in 15 out of 52 women (29%) and 5 out of 23 men (22%) post-RYGB.

This study also shows that the younger the patient, the higher is the probability for suffering from iron deficiency after RYGB [OR per year increase in age=0.94 (95% CI 0.90; 0.99); p=0.012]; this effect was the same in both sexes. Furthermore, patients with an existing iron deficiency before surgery had a much higher probability to suffer from iron deficiency postoperative compared to patients without a baseline iron deficiency [OR=19.49 (95% CI 3.96; 95.99); p<0.01]. The prevalence of iron deficiency was also higher when patients had a vitamin B_{12} deficiency [OR=3.78 (95% CI 1.74; 8.21); p<0.01], both the moments before and after RYGB.

The risk of developing iron deficiency post-RYGB increased over time [OR=1.06 (95% CI 1.03; 1.08) for 1 month increase; p<0.0001]; this trend was the same in both sexes. The prevalence of iron deficiency was considerably increased from 24 months after surgery onwards. Figure 8 shows the predicted probability of ferritin deficiency by time after the surgery.

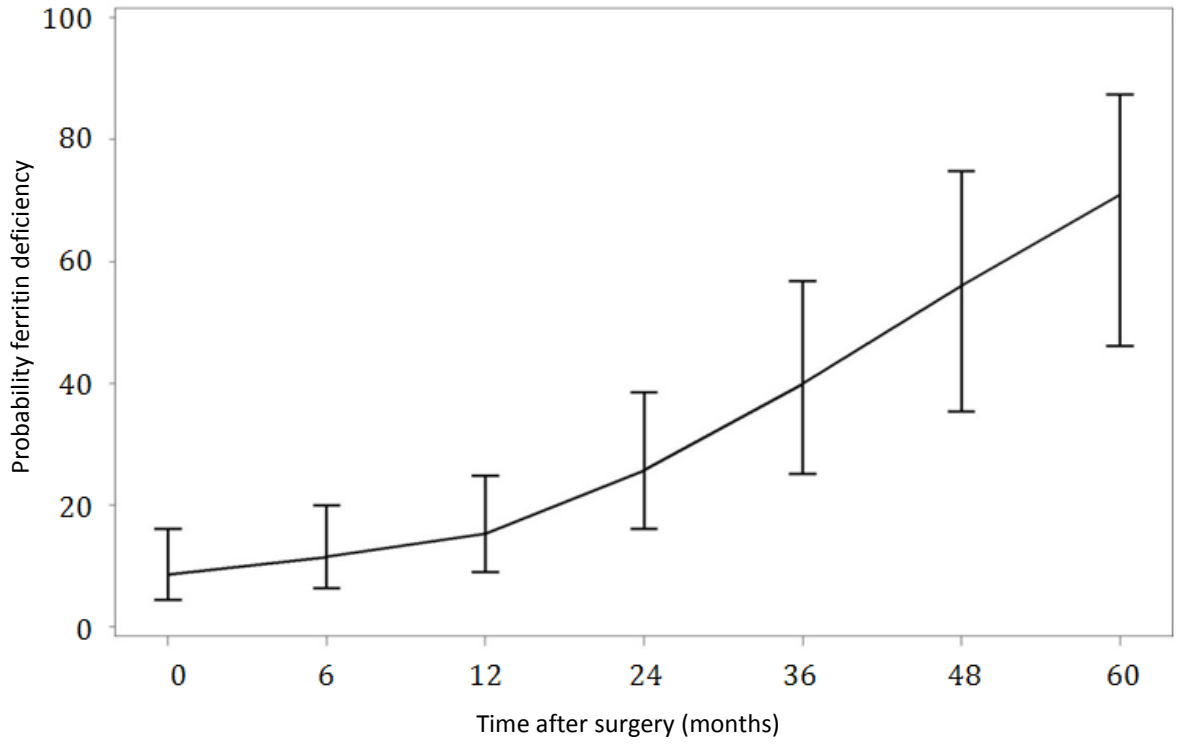

Figure 8: Predicted probability of ferritin deficiency over time (with indication of 95% confidence interval)

There was no evidence for a relationship between the probability of developing iron deficiency and the amount of weight loss [OR=0.98 (95% CI 0.96; 1.01); *p*=0.13] or vitamin D deficiency [OR=0.60 (95% CI 0.20; 1.80); *p*=0.36]. When the group of patients who did take proton pump inhibitors or H_2 antagonists was compared with a group of patients who did not take any of this medication, no difference was found in developing iron deficiency [OR=2.27 (95% CI 0.19; 27.52); *p*=0.52]. An overview of the different associations is shown in Table 18.

Table 18: Association between the development of iron deficiency and other parameters

Parameters	**OR**	**95% Confidence interval**	***p*-value**
Gender: male versus female	0.18	0.04 - 0.73	0.0017
Age: 1 year increase	0.94	0.90 - 0.99	0.012
Baseline ferritin deficiency	19.49	3.96 - 95.99	0.0004
Vitamin B_{12} deficiency	3.78	1.74 - 8.21	0.0009
Time after surgery: increase by month	1.06	1.03 - 1.08	<0.0001
Weight loss	0.98	0.96 - 1.01	0.1271
Vitamin D deficiency	0.60	0.20 - 1.80	0.3620
Consumption of antacids: presence versus absence	2.27	0.19 – 27.52	0.5179

5.3.2 Oral challenge test

An oral iron challenge test has been performed in 23 patients (1 men and 22 women) suffering from iron deficiency post-RYGB (mean age 44 years, SD 11 years) with a mean ferritin of 7.7 µg/L. As shown in Figure 9, there was only one patient who showed sufficient iron absorption (change in serum iron concentration > 80 µg/dL). In four patients, the difference in serum iron concentration before and after the administration of the oral iron supplement was even lower than 20 µg/dL.

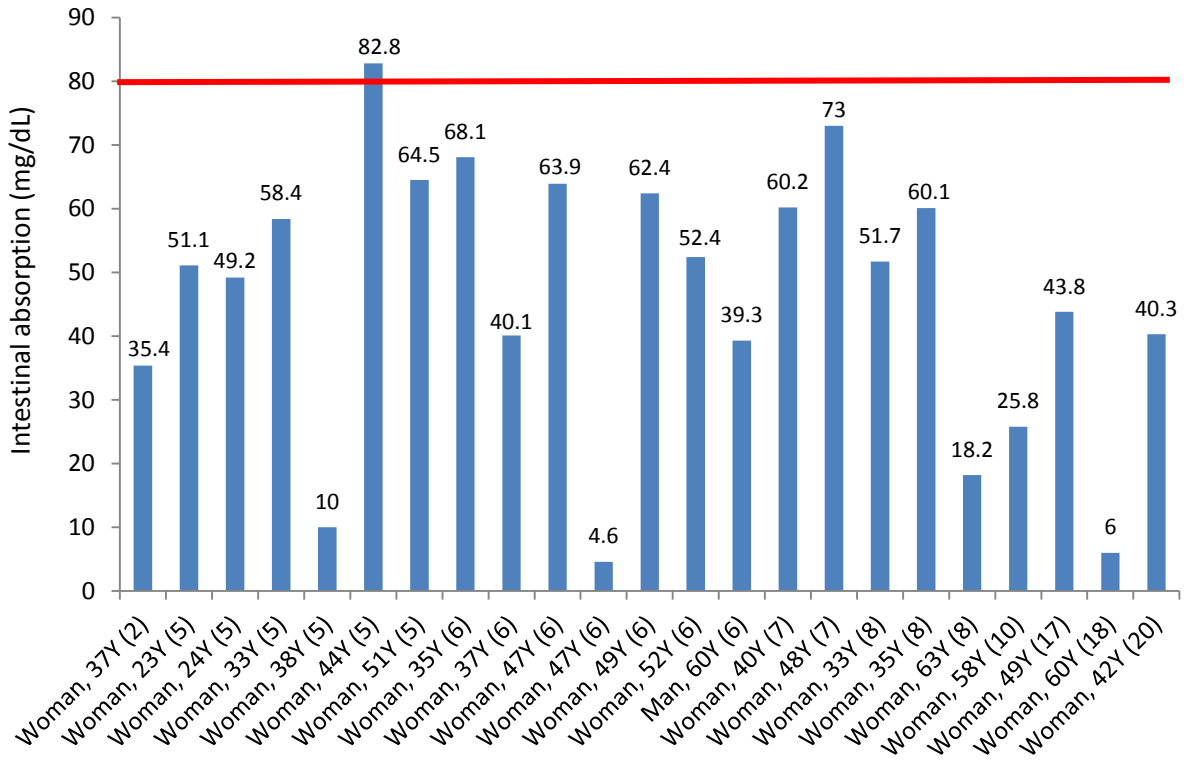

Figure 9: The estimation of the extent of the intestinal iron absorption (expressed as the difference between the highest serum iron concentration after the administration of 100 mg $FeSO_4.7H_2O$ and the serum concentration at *t*=0) for the 23 individuals who received the oral challenge test (including age (years) and serum ferritin levels (microgram per liter) (*between parentheses*)); the red line indicates the minimal change in serum iron concentration to have sufficient iron absorption

5.4 Discussion

5.4.1 Development of iron deficiency and predisposing factors

Thirty-seven percent of patients developed iron deficiency at some point after RYGB. Female gender, young age, poor preoperative iron status, vitamin B_{12} deficiency, and time after surgery were risk factors for the development of iron deficiency after RYGB. In our study, iron deficiency was more prevalent in women than in men, which might be explained by menstrual loss in premenopausal women. Menstruation is often impaired in obese women prior to surgery, but usually resumes after RYGB contributing to iron loss and anemia [146;149].

In line with the results of previous studies, we showed that iron deficiency is not only more prevalent in younger patients before bariatric surgery, but that young age also predisposes to iron deficiency after RYGB [150].

Another interesting finding is that preoperative poor iron status predisposes to iron deficiency after RYGB. This result clearly illustrates the need for an appropriate nutritional evaluation and screening of iron deficiency before RYGB. Consequently, patients should already be treated for iron deficiency before surgery to reduce the probability for developing postoperative iron deficiency and its possible complications [126;151]. We also found that patients with vitamin B_{12} deficiency were also more likely to have iron deficiency. The link between iron and vitamin B_{12} deficiency can be explained by the reduced secretion of gastric acid and the decreased consumption of meat. Previous work of Avinoah et al. indicated that decreased meat consumption is probably the major factor that contributes to the development of iron and vitamin B_{12} deficiency after RYGB [145].

As shown, the risk of developing iron deficiency increases over time: two years post-RYGB, there were significantly more patients with iron deficiency compared to baseline. This is not surprising, as after RYGB, body iron stores gradually decrease [142;151]. As a matter of fact, iron deficiency and subsequent anemia may develop years after the surgery. For that reason, patients require a lifelong

follow-up of hematological and iron parameters, especially menstruating women, pregnant women and adolescents, even after initial repletion of iron [149;152-155].

We found no correlation between developing iron deficiency and the amount of weight loss, which is in line with a study of Avinoah et al. [145]. Furthermore, there was no correlation between developing iron deficiency and vitamin D deficiency or with the use of proton pump inhibitors, H_2 antagonists or antacids. Theoretically, these drugs may increase the risk of developing iron deficiency, as they reduce the acid secretion in the stomach and subsequent iron absorption [126]. A possible explanation for this lack of effect is that after RYGB the stomach is much smaller, which is associated with a reduced acid secretion. Therefore, these drugs may have less impact on acid secretion and iron absorption in patients after RYGB than in patients with a normal size of stomach, but this requires further investigation.

Unfortunately, we have no information on the use of vitamin supplements by the patients studied. This type of information is difficult to collect and can only be done in a prospective trial. Moreover, even when vitamins have been prescribed, compliance is very low.

5.4.2 Absorption of oral iron supplements

In the oral challenge test, only one patient had an increase in plasma iron concentrations of more than 80 µg/dL after the administration of 100 mg iron sulfate. This means that in almost all patients who developed severe iron deficiency post-RYGB, the absorption of oral iron supplements is impaired. Previous studies have also shown that the absorption capacity for iron is reduced after RYGB and that oral iron supplements are often inadequate to correct iron deficiency [152-155]. However, to our knowledge, this is the first report of oral iron absorption tests in patients with severe iron deficiency after RYGB, demonstrating that the development of iron deficiency post-RYGB is largely due to an insufficient absorption of iron. Therefore we support the advice from other papers to switch oral iron supplements to parenteral iron therapy with iron dextran, ferric gluconate, or ferric sucrose if oral treatment is ineffective to correct iron deficiency [152;154]. We do not know

whether the absorption of oral iron supplements is also impaired in patients who do not develop iron deficiency after RYGB. Furthermore, a comparison of absorption of iron before and after surgery is not possible with these data, as the oral iron absorption tests have only been performed in patients after RYGB. So, further research on the absorption of oral iron is needed in order to determine whether it is effective to treat iron deficiencies post-RYGB initially with oral supplements or whether it is more useful for these patients to be directly treated with an intravenous formulation of iron to correct iron deficiency.

5.5 Conclusions

Iron deficiency is a frequent complication after RYGB, necessitating a good follow-up of these patients. Female gender, young age, preoperative poor iron status, vitamin B_{12} deficiency, and the time post-RYGB are predisposing factors for the development of iron deficiency. Iron supplementation seems essential in this population, but the effect of oral tablets may be limited as absorption of oral iron supplements is insufficient post-RYGB.

CHAPTER 6: DISPOSITION OF IRON GLUCONATE FROM AN EFFERVESCENT TABLET IN OBESE PATIENTS BEFORE AND AFTER GASTRIC BYPASS

Manuscript in preparation

6 DISPOSITION OF IRON GLUCONATE FROM AN EFFERVESCENT TABLET IN OBESE PATIENTS BEFORE AND AFTER GASTRIC BYPASS

Background: Roux-en-Y gastric bypass (RYGB) is associated with weight loss and improvement of comorbidities. Nonetheless, these patients have an increased risk for the development of nutritional deficiencies. The objective of this study was to evaluate the disposition of iron gluconate from an effervescent tablet before and after RYGB.

Methods: A single-dose pharmacokinetic study with an effervescent tablet containing 695 mg of iron gluconate as, equivalent to 80 mg Fe^{2+} (Losferron®), was performed before and six to eight months after RYGB in patients with low iron status. Before oral administration, blood was collected for the determination of serum concentrations of iron, hemoglobin, transferrin, transferrin saturation, ferritin, C-reactive protein and hepcidin. After oral administration, blood samples were collected at 15, 30, 60, 90 minutes and 2; 2.5; 3; 3.5; 4; 5; 6; 7; 8; 9; 10 and 24 hours to determine the serum concentration of iron. The $AUC_{0\text{-}24h}$, C_{max} and T_{max} were adjusted for baseline and compared before and after RYGB.

Results: By comparing the $AUC_{0\text{-}24h}$, C_{max} and T_{max} before and after RYGB, no significant differences were observed. Hemoglobin and CRP concentrations were significantly decreased after surgery compared to baseline. The iron status markers, transferrin, TSAT, ferritin and hepcidin were not statistically significant different after RYGB compared to preoperative.

Conclusions: The oral exposure of iron gluconate from an effervescent tablet was not significantly altered in post-RYGB patients with low iron status. The liquid formulation eliminates the need for drug disintegration and dissolution.

6.1 Introduction

The number of Roux-en-Y Gastric Bypasses (RYGB) that are performed, is increasing as the prevalence of obesity has reached epidemic proportions. Bariatric surgery is currently the only available treatment leading to major and sustainable weight reduction in morbid obese patients (BMI ≥ 40 kg/m² or BMI ≥ 35 kg/m² with obesity-related diseases) [141;156]. RYGB results in an altered anatomical structure of the gastrointestinal tract by reducing the gastric capacity and bypassing the duodenum and the proximal jejunum. Changes to the anatomical structure of the gastrointestinal tract following RYGB can alter the pharmacokinetics of a given drug by an increase in gastric pH (due to gastric resection and the widespread use of antacid medication following surgery), a reduction of the small intestinal surface area available for absorption as well as a potential bypass of intestinal regions especially important for absorption (i.e. high/low abundance of transporters and/or drug metabolizing enzymes) [58;59]. All of the above stated changes may impact the absorption of supplements and drugs. Furthermore, the reduced gastric capacity is associated with a reduced food intake after RYGB [48]. Both the reduced intake of food and the impaired absorption of supplements, increase the risk for the development of nutritional deficiencies in patients with a RYGB [11].

The most common nutritional deficiencies after RYGB include iron, vitamin B_{12}, folic acid, vitamin D, and calcium deficiencies. Some of these deficiencies can result in severe complications such as anemia [98;126]. Following RYGB, 20 to 49% of patients develop an iron deficiency. This can be explained by (1) diminished gastric acid secretion, which is necessary for the absorption of iron; (2) reduced intestinal absorption surface, particularly the excision of the duodenum which is the main absorption site of iron; and (3) low tolerance to red meat, a major source of iron [23;157]. Diet and the use of standard multivitamin/mineral supplements are often insufficient to prevent iron deficiency; therefore, patients with RYGB are recommended to use oral iron supplements [14]. However, as shown in previous studies the absorption of iron from oral iron supplement tablets is impaired after RYGB [24;25;158]. Both the type of the micronutrient such as the salt form or amino chelate, and the formulation of the supplement have an influence on the absorption. The effect of the type of

formulation has been shown for calcium: the bioavailability of calcium citrate from an effervescent formulation was superior to a tablet formulation in RYGB patients [136]. Until now, no studies with an effervescent iron supplement have been performed after RYGB and we hypothesized that the disposition of iron from a solution would be no problem in contrast to a tablet formulation. Therefore, the objective of this study was to evaluate the disposition of iron gluconate from an effervescent tablet before and after RYGB.

6.2 Methods

6.2.1 Selection of patients

Fifteen obese patients, who had a low iron status at the preoperative consultation (ferritin < 30 µg/L and/or transferrin saturation < 20%) and had a RYGB surgery planned in the University Hospitals Leuven, were recruited. Patients with a bariatric surgery history and pregnant or lactating women were excluded from the study. Patients with a positive *Helicobacter pylori* screening before surgery were also excluded as this infection can reduce iron absorption [159]. None of the participants was a tobacco user. In the recruited patients, a laparoscopic gastric bypass with an alimentary limb of 120 cm and a small gastric pouch was performed by the same surgeon according the same procedure. This study was approved by the Medical Ethics Committee of the University Hospitals Leuven (ML8433) and all patients gave written informed consent. The EudraCT number is 2012-001244-22.

6.2.2 Study design and procedure

A single-dose pharmacokinetic study with 695 mg of iron gluconate (further referred to as 'iron'), equivalent to 80 mg Fe^{2+} (Losferron®) was performed in all patients before and six to eight months after RYGB (on average 6.4 months (SD 0.6); further referred to as 'after RYGB'). Losferron® is an effervescent tablet formulation.

Following an overnight fast of at least 10 hours, subjects came to the clinical pharmacology unit of the University Hospitals Leuven. Weight and height of the subjects were measured with calibrated equipment. The weight was measured to the nearest 0.1 kg, with the subjects having an emptied

bladder and wearing indoor clothing with empty pockets and without shoes. BMI (kg/m²) was calculated by dividing the weight (kg) by the square of height (m²). The percentage of excess weight loss was calculated using the formula:

$$\%EWL = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight} - \text{ideal body weight}} \quad [102]$$

A Dual Energy X-ray Absorptiometry (DXA) was performed to measure the amount of body fat.

After the insertion of an intravenous catheter, the subjects ingested 695 mg of iron gluconate which was dissolved in 150 mL of water, completely effervesced with minimal carbonation. Before oral administration, blood samples were collected for the determination of the serum concentration of iron, hemoglobin, transferrin, transferrin saturation (TSAT), ferritin, C-reactive protein (CRP) and hepcidin. Iron, hemoglobin, transferrin, ferritin and CRP concentrations were determined by the Clinical Laboratory of the University Hospitals Leuven, Belgium. TSAT was calculated using the following formula:

$$\text{TSAT}\ (\%) = \frac{\text{Iron}\left(\frac{\mu g}{dL}\right) * 100}{\text{Transferrin}\left(\frac{g}{L}\right) * 1.4}$$

Hepcidin concentrations were determined using the hepcidin-25 (human) Enzyme-Linked mmunosorbent Assay (ELISA) kit from Peninsula Laboratories International, Inc.

After oral administration of iron, blood samples were collected at 15, 30, 60, 90 minutes and 2; 2.5; 3; 3.5; 4; 5; 6; 7; 8; 9; 10 and 24 hours to determine the serum concentration of iron. After each collection, the blood sample was immediately sent to the Clinical Laboratory of the University Hospitals Leuven, Belgium. In these samples, the serum concentrations of iron were determined by a colorimetric analysis with the ferrozin method (Hitachi/Roche-Cobas c702).

A standardized meal (containing 0.8 mg of iron, determined based on the Belgian Food Composition DataBase - NUBEL) and a standardized snack (containing 0.1 mg of iron) were administered 4 hours and 8 hours, respectively, after the administration of the effervescent tablet. Participants had to consume the entire meal. The use of water was allowed, except for one hour before and four hours after drug administration. During four hours after the administration, the patients had to remain

semi-supine in bed. After the 10h-blood sample, the subjects were discharged and they had to return the next morning fasted for the 24-h blood sampling.

The included patients were asked to stop multivitamin and iron supplements, proton pump inhibitors, H_2-receptor antagonists and antacids during the week preceding the study, as their use can influence the absorption of the iron supplement.

6.2.3 Data analysis

The oral exposure of iron gluconate was estimated by changes in serum iron concentration. For that purpose, serum iron concentrations after administration of the iron supplement were adjusted for the basal iron concentration by dividing serum iron concentration after administration by serum iron concentration before administration. Subsequently, the $AUC_{0\text{-}24h}$ concentration-versus-time curves were determined using the linear trapezoidal rule.

To compare the characteristics, status markers and pharmacokinetic parameters ($AUC_{0\text{-}24h}$, C_{max} and T_{max}) of the participants before and after RYGB, a paired *t*-test was performed when the assumption for normal distribution of the data was accepted (Shapiro-Wilk test). The $AUC_{0\text{-}24h}$ and C_{max} were transformed with the logarithm function to achieve normality. If no normality was achieved (ferritin), a Wilcoxon signed-rank test was performed. A linear mixed model was used to compare the change in plasma iron concentration after oral iron administration before and after RYGB. All results are presented as estimated mean±SEM, unless otherwise mentioned. Statistical significance was set at $p<0.05$. The data were analyzed with SPSS Statistics 22.

6.3 Results

Thirteen volunteers (10 female, 3 male) were recruited and participated in the single-dose pharmacokinetic study before and after RYGB. The characteristics of the subjects and the iron and hematological parameters before and after RYGB are shown in Table 19. Weight, BMI and fat mass percentage were significantly decreased post-RYGB. The mean age at the time of the surgery was 45.6 (SD 11.6) years. Five patients were taking multivitamin and -mineral supplements and six

patients used a specific iron supplement at the time of the postoperative study, but were asked to stop the intake one week before the study. Before surgery, nobody was taking an iron containing supplement at the time of the study.

Table 19: Characteristics of patients, shown as mean±SD

Parameter	Before RYGB	After RYGB
Weight (kg)	111.8±12.7	82.2±9.1*
Height (m)	1.7±0.1	1.7±0.1
Body mass index (kg/m²)	39.9±3.1	29.3±2.1*
Fat mass (%)	42.4±6.3	34.3±6.9*
Hemoglobin (g/dL)	13.5±0.8	12.5±1.1*
Transferrin (g/L)	2.7±0.3	2.5±0.4
Transferrin saturation (%)	17.3±5.2	20.2±6.6
Serum ferritin (µg/L)	91.8±68.6	136.1±176.9
Hepcidin (ng/mL)	32.0±30.1	28.3±21.3
CRP (mg/L)	4.1±3.7	3.0±3.7*

*p<0.05 compared to baseline

Hemoglobin concentration was significantly decreased after surgery compared to baseline. The iron status markers, transferrin, TSAT, ferritin and hepcidin were not statistically significant different after RYGB compared to preoperative. CRP was significantly decreased.

The pharmacokinetic parameters are shown in Table 20. No significant differences regarding the $AUC_{0\text{-}24h}$ adjusted to baseline was observed (paired *t*-test: *p*=0.39). The peak concentration of iron was reduced from 1.9 µg/dL to 1.6 µg/dL after RYGB; T_{max} was 3.0±0.7 h before RYGB and 2.7±0.5 h post-RYGB. No statistically significance was reached for both pharmacokinetic parameters.

The serum iron changes after administration of the oral iron supplement are shown in Figure 10. Overall, there was no statistically significant difference in total iron plasma concentration before and

after surgery (*p*=0.52). After RYGB, the plasma iron concentration, as a response to the administration of iron, had the tendency to be lower, but no statistically significance was reached.

Table 20: Pharmacokinetic results after oral administration of Losferron®, based on the adjusted concentrations, before and after RYGB shown as mean±SEM

Parameter	Before RYGB	After RYGB	*p*-value
$AUC_{0\text{-}24h}$ (µg/dL*h)	31.4±3.1	28.2±2.6	0.39
C_{max} (µg/dL)	1.9±0.3	1.6±0.2	0.32
T_{max} (h)	3.0±0.7	2.7±0.5	0.76

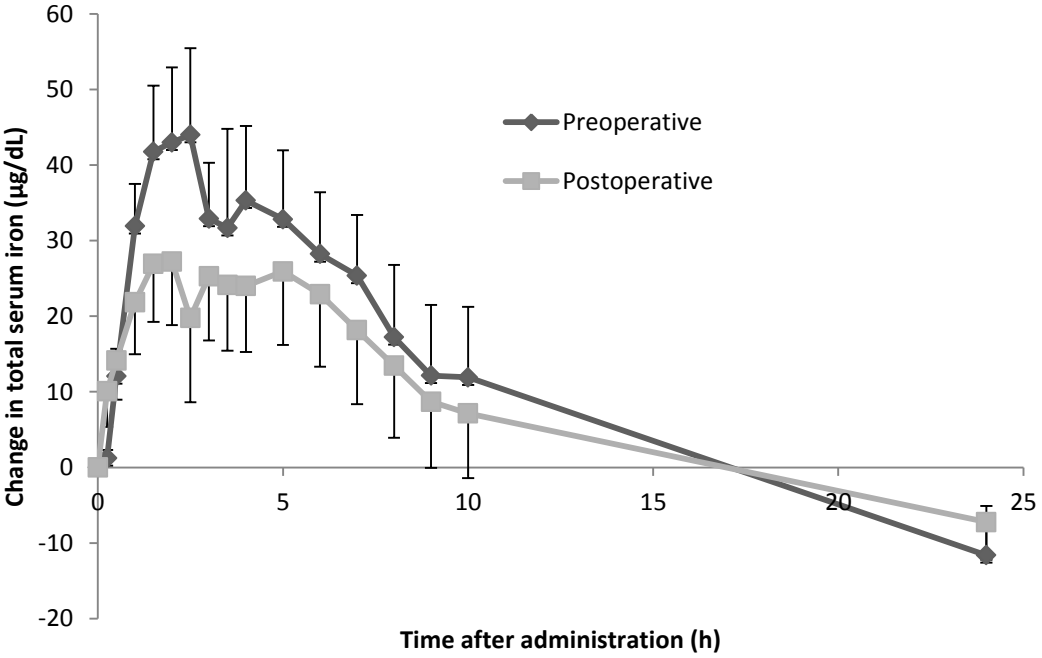

Figure 10: Serum iron change during 24h after oral administration of iron gluconate before and after RYGB, shown as mean±SEM

6.4 Discussion

A lot of patients develop iron deficiency after RYGB and need to take iron supplements. This study aimed to evaluate the disposition of oral iron gluconate from an effervescent tablet before and after RYGB. We have shown that in patients who have undergone a RYGB the oral exposure of iron from an effervescent tablet is not significantly different from the preoperative situation. This is different as

reported in most previous studies regarding iron absorption after RYGB. In this study, we used iron gluconate salt and an effervescent tablet. Both the type of iron and the formulation can influence the absorption.

It is known that the formulation of a drug supplement can influence the extent of absorption. A study performed by Sakhaee et al. [136] demonstrated that the bioavailability of calcium citrate from an effervescent tablet was superior compared to the bioavailability from a tablet formulation. Therefore, in this study, we have also chosen for an effervescent tablet resulting in a liquid formulation for administration which eliminates the need for tablet disintegration and drug dissolution and subsequently could increase absorption [57]. The fact that a solution is being administered may explain that the oral exposure is comparable before and after RYGB. This may suggest that the formulation of the iron supplement in the current study is the main factor contributing to the smaller decrease in iron absorption after RYGB compared to previous studies performed with a tablet formulation. Up until now, we do not know to what extent gastric contractions and gastric motility are altered after RYGB. Both factors have an important impact on drug disintegration from tablets. The advantage of administration of a solution is that its absorption is not dependent on these factors.

In our study, iron gluconate was administered to the participants. Previously, Rhode et al. [132] have already performed iron absorption tests with iron gluconate in patients after gastric bypass. Normal absorption, which was defined as more than 100% change in serum iron concentration over 3 hours after administration, was observed in 36 of the 55 patients. The patients with normal absorption had a higher incidence of anemia and had lower serum ferritin concentrations compared to those with low absorption. The fact that iron deficiency results in a facilitation of iron absorption [160], and that we only included patients who suffered from iron deficiency, could further explain our results. The iron status was not improved after RYGB in these patients as no significant differences in ferritin, TSAT and hepcidin were observed, which could be explained by the preoperative poor iron status.

The type of salt could also have contributed to the improved absorption of iron post-RYGB, compared to other studies. Previous studies with other iron salts have shown an impaired absorption of iron post-RYGB [24;26]. However, we need to take into account that all these studies have a different design and were performed with supplements in tablet formulation. Ruz et al. [24] have performed iron absorption tests with ferrous ascorbate using radioactive labeled iron. They have shown that the iron absorption was significantly reduced in women 6 months post-RYGB to 40.3% of baseline values. No further significant changes of the absorption were observed 12 and 18 months post-RYGB. Rosa et al. [26] have performed iron tolerance tests (*n*=9♀) with iron sulphate (15 mg elemental iron) before and 3 months after RYGB. During 4 hours after administration, no statistically significant differences in total iron plasma response were shown, only a delayed response, even though 6 of the 9 patients presented a mean decrease in AUC of 51%. The first hour after administration, the plasma concentration of iron was significantly lower after surgery compared to the situation before surgery [26]. Previously, we have also performed oral iron absorption tests with 100 mg of iron sulphate in patients post-RYGB and only one patient out of 23 patients showed sufficient iron absorption [158].

Furthermore, we need to be aware that the absorption before surgery also could have been impaired, since a negative correlation has been identified between iron absorption and BMI [161]. The low-grade inflammation induced by obesity reduces iron absorption as inflammation is a negative regulator for iron absorption [128;161]. The inflammation status improved in the participants after RYGB as the CRP concentration was significantly decreased post-RYGB [131]. Hence, iron absorption after RYGB was less or not inhibited by inflammation.

Nonetheless, RYGB is associated with a reduced gastric capacity resulting in reduced gastric acid secretion that is necessary for the conversion of Fe^{3+} to the absorbable form Fe^{2+}; and with a bypass of the proximal part of the intestine, which is the main absorption site of iron [24]. Hence, we can state that the absorption of iron is probably impaired both pre- and post-RYGB, but that there is a switch regarding the causes of this reduced absorption. In order to avoid problems resulting in

reduced iron absorption after RYGB, a formulation change to an effervescent tablet provided promising results.

Finally, we need to take into account that the body does not have a specific mechanism to eliminate iron. Long-term use of high-dose iron supplements with inadequate monitoring can result in iron overload [162]. Therefore, monitoring of non-transferrin bound iron could be useful as it has a key role in iron toxicity. Circulating non-transferrin bound iron is present in the bloodstream independently of transferrin. It can appear independently of the presence of available binding sites on transferrin on the condition that rate of iron influx into plasma exceeds the rate of iron acquisition by transferrin [134;135].

The absorption of iron gluconate from an effervescent tablet was not significantly different before and after RYGB after a single dose administration. It would be interesting to explore long-term effects.

In the current study, we have only tested an effervescent tablet. To determine whether the formulation has indeed a large impact on the iron absorption after RYGB, it would be interesting to compare the disposition of the same iron salt from a tablet and from an effervescent tablet formulation. Additionally, the strength of gastric contractions could be measured to determine to what extent gastric mixing is altered after RYGB. This could be done by high-resolution manometry (HRM) [163]. Furthermore, it could be interesting to test the iron absorption at different time points after RYGB as intestinal adaptation can take place. This has already been shown for carbohydrate absorption with an up-regulation of intestinal glucose transporters after RYGB surgery [137]. Furthermore changes in gut microbiota after RYGB can play a role in nutrient absorption [138]. Further research regarding nutrient absorption is needed.

6.5 Conclusions

The oral exposure of iron gluconate from an effervescent tablet was not altered in post-RYGB patients with iron deficiency. This could be explained by the type of salt and the type of formulation

that was chosen in this study. A liquid formulation eliminates the need for drug disintegration and dissolution and subsequently may increase absorption. Hence, the choice of formulation of supplements needs to be considered in patients with RYGB.

PART IV: INFLUENCE OF RYGB ON DISPOSITION OF DRUGS

CHAPTER 7: DRUG DISPOSITION AND MODELLING BEFORE AND AFTER GASTRIC BYPASS: IMMEDIATE AND CONTROLLED RELEASE METOPROLOL FORMULATIONS

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Drug disposition and modelling before and after gastric bypass: immediate and controlled release metoprolol formulations

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7 DRUG DISPOSITION AND MODELLING BEFORE AND AFTER GASTRIC BYPASS: IMMEDIATE AND CONTROLLED RELEASE METOPROLOL FORMULATIONS

Background: Roux-en-Y gastric bypass alters the anatomical structure of the GI-tract, which can result in alterations in drug disposition. Therefore, the aim of this study was to evaluate the disposition of metoprolol after oral administration of an immediate and controlled release formulation before and after Roux-en-Y gastric bypass (RYGB) surgery in the same individuals and to validate a physiologically-based pharmacokinetic (PBPK) model for predicting oral bioavailability following RYGB.

Methods: A single-dose pharmacokinetic study of metoprolol tartrate 200 mg immediate release (Lopresor®) and controlled release (Slow-Lopresor®) was performed in 14 volunteers before and six to eight months after RYGB. The observed data were compared with predicted results from the PBPK modelling and simulation of metoprolol tartrate immediate and controlled release formulation before and after RYGB.

Results: After administration of metoprolol immediate and controlled release, no statistically significant difference in the observed $AUC_{0\text{-}24h}$ was shown, although a tendency towards an increased oral exposure could be observed as $AUC_{0\text{-}24h}$ was 32.4% (95% CI 1.36; 63.5) and 55.9% (95% CI 5.73; 106) higher following RYGB for the immediate and controlled release formulation, respectively. This could be explained by surgery related weight loss and a reduced presystemic biotransformation in the proximal GI-tract. The PBPK modelling and simulation predicted values were similar to the observed data, confirming its validity.

Conclusions: The disposition of metoprolol from an immediate release formulation and a controlled release formulation was not significantly altered after RYGB; there was a tendency to an increase, which was also predicted by PBPK modelling and simulation.

7.1 Introduction

Over the last decades, the prevalence of obesity has increased dramatically [1]. This has led to an increased demand for bariatric surgery, especially Roux-en-Y gastric bypass (RYGB), which is the only available treatment leading to major and sustainable weight reduction in morbid obese patients (BMI ≥ 40 kg/m² or BMI ≥ 35 kg/m² with obesity-related diseases) [141]. RYGB results in an altered anatomical structure of the gastrointestinal tract by reducing the gastric capacity and bypassing the duodenum and the proximal jejunum. Changes to the anatomical structure of the gastrointestinal tract following RYGB can alter the pharmacokinetics of a given drug by an increase in gastric pH (due to gastric resection and the widespread use of antacid medication following surgery), a delayed inlet of bile acids, a reduced small intestinal surface area available for absorption and a potential bypass of intestinal regions with high abundance of drug metabolizing enzymes [58;59]. All of the above stated changes may impact oral drug absorption and bioavailability. However, the extent to which absorption is hampered for a specific drug, or class of drugs, remains unknown. The few studies that have been conducted illustrate that a trend in oral drug exposure before to after RYGB is not easy to predict. On the one hand, oral exposure can be reduced following RYGB, as reported for azithromycin [164]; on the other hand, it can remain unaltered, as reported for levothyroxine, or be increased, as for metformin [72;79]. A systematic approach to study the influence of RYGB on oral drug exposure is lacking as previously conducted studies vary in design and are poorly standardized based on how surgery is conducted, which makes the results difficult to compare.

Drug substances can be classified according to their solubility and permeability, which forms the basic concept of the Biopharmaceutical Classification System (BCS) [165]. Based on its absorption characteristics, we have chosen to investigate the influence of RYGB on metoprolol, a BCS class I compound, which is characterized by a high solubility and high permeability. Metoprolol is a β_1-blocker, and β-blockers are widely used cardiovascular drugs. Metoprolol (pK_A = 9.18) is known to cross the intestinal mucosa by passive diffusion [166]. It is mainly metabolized by cytochrome P450 2D6 (CYP2D6) and in healthy volunteers, the half-life of metoprolol amounts to 3-4 h [167;168]. For the

investigation of the influence of RYGB surgery on the disposition of metoprolol, we have chosen for an immediate release formulation and for a controlled release formulation; so far, controlled release formulations have never been studied in RYGB patients. It is generally advised to avoid formulations with a controlled release after bariatric surgery, but this advice is purely eminence based [57]. An additional aim of this study was to serve as an ongoing validation of a previously developed physiologically-based pharmacokinetic (PBPK) model [60] for predicting oral drug exposure following RYGB in order to validate its use for dose adjustments following surgery. This way, potential dangerous over- or underdosing of drugs can be avoided, which is especially a risk for drugs with a narrow therapeutic range.

Hence, this paper reports on two types of investigations: (1) evaluation of the oral pharmacokinetic parameters of metoprolol tartrate, a β_1-blocker belonging to class I of the BCS (high solubility/high permeability), immediate and controlled release in obese patients before and after RYGB; (2) comparison of the *in vivo* data to the predictions from the PBPK modelling of metoprolol tartrate immediate and controlled release formulation before and after RYGB.

7.2 Methods

7.2.1 Selection of patients

For this study (EudraCT number is 2012-001244-22), 14 obese patients with a planned RYGB surgery at the University Hospitals Leuven, Belgium, were recruited. Patients who had previously undergone bariatric surgery or who had renal and hepatic impairment were not included in the study. Pregnant and breastfeeding women were also not included. RYGB surgery was performed in all recruited patients by the same surgeon. In brief, the jejunum was divided 30 cm from the ligament of Treitz and anastomosed to a 30 mL proximal gastric pouch. The jejunum was reanastomosed 120 cm distally to the gastrojejunostomy. All mesenteric defects were closed. The study was approved by the Medical Ethics Committee of the University Hospitals Leuven (ML8433) and all patients gave written informed consent.

7.2.2 Study design and procedure

A single-dose pharmacokinetic study of metoprolol tartrate (referred to as metoprolol) 200 mg immediate release (Lopresor®) and controlled release (Slow-Lopresor®) was performed before and six to eight months after RYGB (on average 6.6 months [SD 0.63]; further referred to as six months after RYGB). Both formulations were tested in all patients before and after RYGB, with an interval of at least 5 days between administrations of the two formulations. The relative extent of oral exposure of metoprolol from both formulations was estimated by the determination of the area under the curve ($AUC_{0\text{-}24h}$), the peak plasma concentration of metoprolol after oral administration (C_{max}) and the time to reach peak concentration (T_{max}). The $AUC_{0\text{-}24h}$ reflects drug absorption and drug elimination; in this paper we have mainly focused on drug absorption as a RYGB mainly influences the absorption through the formation of a gastric pouch and bypass of the proximal part of the small intestine.

Following an overnight fast of at least 10 hours, subjects came to the clinical pharmacology unit of the University Hospitals Leuven. Weight and height of the subjects were measured with calibrated equipment. The weight was measured to the nearest 0.1 kg, with the subjects having an emptied bladder and wearing indoor clothing with empty pockets and without shoes. BMI (kg/m^2) was calculated by dividing the weight (kg) by the square of the height (m^2). A Dual Energy X-ray Absorptiometry (DXA) was performed to measure the amount of body fat mass [169].

After the insertion of an intravenous catheter, the subjects ingested 200 mg of metoprolol (2 tablets of Lopresor® 100 mg or 1 tablet of Slow-Lopresor® 200 mg) with 150 mL of water. The tablets were taken without being broken or crushed. After oral administration, blood samples were collected into heparinized tubes at 15; 30; 60; 90 minutes and 2; 2.5; 3; 3.5; 4; 5; 6; 7; 8; 9; 10 and 24 hours. The blood samples were centrifuged immediately after collection (1800 g, 10 min, 4°C) and plasma samples were stored at -20°C until analysis. At each time point, the systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate were determined with Omron, Model M6, Digital automatic blood pressure monitor, Intellisens™.

A standardized meal and a standardized snack were administered 4 hours and 8 hours after drug administration, respectively. Participants had to consume the entire meal. The use of water was allowed ad libitum, except for one hour before and four hours after drug administration. During the first 4 hours after administration of metoprolol, the patients had to remain semi-supine in bed. After the 10h-blood sample, the subjects were discharged and they had to return the next morning for the 24-h blood sampling. As proton pump inhibitors, H_2-receptor antagonists and antacids could influence the absorption of drugs, the recruited patients were asked to stop these drugs during the week preceding the study. Other prescription drugs were checked to verify that there were no pharmacokinetic interactions with the study drug. The morning of the study, the patients were not allowed to take their medication.

All procedures were in accordance with the ethical standards of the Medical Ethics Committee of the University Hospitals Leuven.

7.2.3 HPLC analysis

The determination of the concentration of metoprolol was performed by a validated HPLC method with fluorescence detection (ex.271 nm, em.302 nm; Waters 2475 Multiwavelength Fluorescence Detector). Metoprolol was extracted after adding 1.25 mL of 0.2 M HCl, 0.10 mL of 200 nM propranolol (as internal standard), 0.50 mL of 2 M NaOH and 10.00 mL of CH_2Cl_2 to 0.50 mL of plasma by repeatedly vortexing. After extraction for one minute, it was centrifuged (4000 rpm, 10 min, 4°C) and the supernatant was removed. The remaining organic solution was evaporated and the residue was dissolved in MeOH. This solution was evaporated again and resuspended in 0.15 mL of transport medium, which was injected into the HPLC system, equipped with an Alliance 2695 separations module and a Novapak C-18 column under radial compression (Waters, Milford, MA, USA).

A gradient run was performed with 25 mM acetate buffer pH 3.5:methanol (51:49 v/v) during the first three minutes, followed by six minutes 25 mM acetate buffer pH 3.5:methanol (45:55 v/v). Then the column was rinsed with acetonitrile:water (90:10 v/v). The flow rate amounted to 1.10 mL/min

resulting in a retention time of 4.2 min and 8.2 min for metoprolol and the internal standard, respectively.

Calibration curves were made based on a stock solution of metoprolol in dimethyl sulfoxide and linearity was observed between 1337 ng/mL and 5 ng/mL. The intra-day accuracy and precision errors were 3.3% and 6.4%, respectively, for a concentration of 535 ng/mL (corresponding to 2000 nM) and 3.0% and 8.6%, respectively, for a concentration of 53 ng/mL (corresponding to 200 nM). The inter-day accuracy and precision errors were 6.1% and 1.0%, respectively, for a concentration of 535 ng/mL, and 4.8% and 3.5%, respectively, for a concentration of 53 ng/mL.

7.2.4 Data and chapter analysis

The $AUC_{0\text{-}24h}$ of the concentration-versus-time profiles was determined using the linear trapezoidal rule. Data are presented as mean (95% confidence interval, CI), unless otherwise mentioned. To evaluate the effect of RYGB on the pharmacokinetic parameters of metoprolol, $AUC_{0\text{-}24h}$, C_{max} and T_{max} obtained before and after surgery were compared. The paired data were analyzed with SPSS Statistics 22, performing a paired *t*-test as the assumption for normal distribution of the data was accepted (Shapiro-Wilk test). The $AUC_{0\text{-}24h}$ of the controlled release formulation was transformed with the logarithmic function to achieve normality. For the data analysis of T_{max}, a Wilcoxon signed-rank test was performed as normality was not achieved. Multiple linear regression analysis was performed to control for correlation on the oral exposure of metoprolol including gender, age, BMI, fat percentage as measured by DXA, weight loss, systolic and diastolic blood pressure and heart rate were included. No significant correlations were identified for $AUC_{0\text{-}24h}$; no adjustments for these factors were made. To compare the baseline pharmacodynamic parameters a paired *t*-test was performed and the comparison of the pharmacodynamic profiles was carried out using a linear mixed model. Statistical significance was set at $p<0.05$.

7.2.5 Physiologically-based pharmacokinetic modelling and simulation

PBPK modelling and simulation was employed using the previously developed and validated RYGB PBPK absorption model, based on the obesity model by Ghobadi et al. (2011) available in the Simcyp Simulator, considering obesity related changes in drug disposition [170]. The RYGB PBPK absorption model was coupled to the minimal PBPK model incorporated into the Simcyp Simulator version 13.1, in order to elucidate the potential mechanism behind the observed trend in oral drug exposure of metoprolol immediate and controlled release formulation before to after RYGB [60;171;172]. Metoprolol immediate and controlled release compound files were developed based on the pre-validated metoprolol compound as supplied in the Simcyp compound library. Distributional parameters describing a two-compartmental distribution behaviour (V_{ss}, V_{sac}, k_{in}, k_{out}) were estimated based on intravenous data from Regardh et al. [173] using the parameter estimation toolbox, obtaining the following estimates: 2.58 L/kg, 1.89 L/kg, 5.75 h^{-1} and 5.09 h^{-1} for V_{ss}, V_{sac}, k_{in} and k_{out}, respectively. Clearances via cytochrome P450 isoforms 3A4 and 2D6 were estimated using the retrograde model, back-calculating intrinsic clearance (CL_{int}) from intravenous clearance assuming a 7% contribution by CYP3A4 (Simcyp Simulator v13.1). *In vitro* release profiles of metoprolol immediate and controlled release formulations were obtained from Oosterhuis et al. [174] and Polli et al. [175] and were fitted to a Weibull function using Matlab R2012a (Mathworks, Natick, MA, USA). For the immediate release metoprolol formulation the Weibull function describing the dissolution profile derived from simulated gastric fluid was directly implemented into the Simcyp Simulator. Dissolution of the controlled release formulation was scaled by *in vitro-in vivo* correlation (IVIVC) based on fast, medium and slow extended release profiles and plasma concentration-time data as reported by Eddington et al. [176]. The IVIVC produced a correction factor of 0.93 using the module in Simcyp Simulator v13.1. The RYGB absorption model was adapted as per Darwich et al. [171] in order to account for population-specific demographics (body weight, height, age and gender) before and after RYGB, and surgical dimensions. Furthermore, oral bioavailability (F_{oral}) was calculated using the following equation:

$$F_{oral} = F_A * F_G * F_H$$

where F_A stands for fraction of drug absorbed; F_G is the fraction of drug escaping gut wall metabolism and F_H stands for the fraction of drug entering the portal vein escaping first pass metabolism in the liver.

7.3 Results

In this study, we recruited 14 patients (10 women, 4 men) with a mean age of 44.4 years (95% CI 38.0; 50.7). The main characteristics of the participants are shown in Table 21.

The observed and predicted pharmacokinetic data are summarized in Table 22 and the observed concentration-time profiles are shown in Figure 11.

Table 21: Characteristics of the participants, shown as mean (95% CI)

Patient demographics	**Before RYGB**	**After RYGB**
Weight (kg)	110.4 (103.7; 118,2)	80.4 (74.6; 87.4)*
BMI (kg/m²)	38.8 (37.0; 40.3)	28.3 (26.5; 29.9)*
% weight loss	0	61.7 (54.3; 69.2)*
Fat percentage (%)	43.1 (38.8; 46.6)	32.7 (28.5; 37.0)*

Table 22: Pharmacokinetic results and predicted results for the immediate (IR) and controlled release (CR) formulations of metoprolol before and after surgery, shown as mean (95% CI)

	AUC_{0-24h} (ng/mL*h)	**Predicted AUC_{0-24h} (ng/mL*h)**	**C_{max} (ng/mL)**	**Predicted C_{max} (ng/mL)**	**T_{max} (h)**	**Predicted T_{max} (h)**
Metoprolol IR preoperative	2373 (1490; 3256)	2169	404 (288; 520)	348	1.36 (1.03; 1.69)	1.72
Metoprolol IR postoperative	3206 (1756; 4656)	2521	532 (323; 741)	465	1.25 (0.89; 1.61)	1.25
Post- vs pre-RYGB	Ratio 1.35	Ratio 1.16	Ratio 1.32	Ratio 1.34	Difference 0.11	Difference 0.47
Metoprolol CR preoperative	1917 (1023; 2812)	1760	-	-	-	-
Metoprolol CR postoperative	2333 (1372; 3293)	2020	-	-	-	-
Post- vs pre-RYGB	Ratio 1.22	Ratio 1.15	-	-	-	-

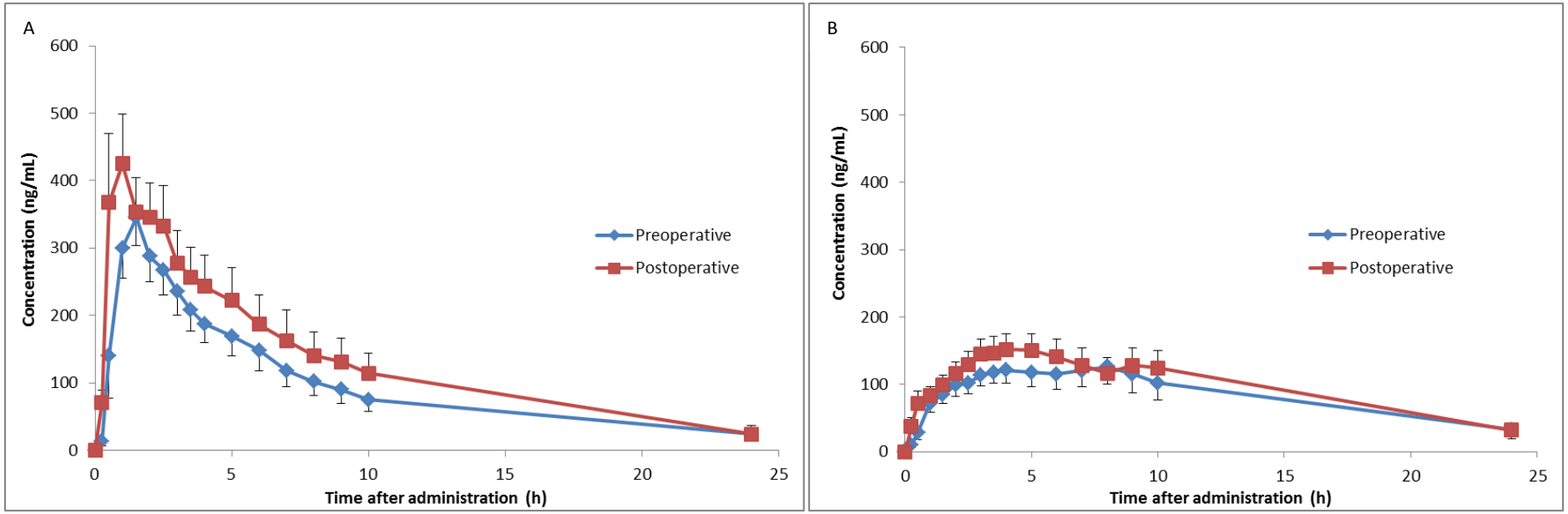


Figure 11: Observed plasma concentration-time profiles of metoprolol over 24h after the administration of an immediate release dosage form before and after RYGB (A) and a controlled release dosage form before and after RYGB (B), shown as mean concentration±SEM (*n*=14)

The $AUC_{0\text{-}24h}$ of metoprolol immediate release was 32.4% (95% CI 1.36; 63.5) higher 6 months following RYGB than before surgery. However, this difference was not statistically significant (paired *t*-test: *p*=0.07). C_{max} for metoprolol immediate release tended to be 29.0% (95% CI -1.86; 59.8) higher after RYGB, but this difference did not reach statistical significance (paired *t*-test: *p*=0.07). The time to reach maximum plasma concentration was also not statistically significant different, but with a trend of T_{max} being shorter after the operation: T_{max} decreased from 1.36 h (95% CI 1.03; 1.69) to 1.25 h (95% CI 0.89; 1.61) (Wilcoxon: *p*=0.68). These trends were similar to the predicted data from the PBPK simulation and modelling: $AUC_{0\text{-}24h}$ and C_{max} were also higher after RYGB by 16.0% and 34.0%, respectively; T_{max} was shorter with a decrease from 1.72 h to 1.25 h after RYGB. The half-life increased from 4.2 h (95% CI 3.1; 5.3) before RYGB to 4.9 h (95% CI 3.2; 6.6) after RYGB; this difference was not significant.

After administration of the metoprolol controlled release formulation, no statistically significant difference in $AUC_{0\text{-}24h}$ of metoprolol was observed, although a tendency towards increased oral exposure could be observed after RYGB as the $AUC_{0\text{-}24h}$ after oral administration of the controlled release formulation was 55.9% (95% CI 5.73; 106) higher than before RYGB (paired *t*-test: *p*=0.30). The same observation was made for the predicted data. In Figure 12 the data from the *in vivo* pharmacokinetic study and the predicted data for metoprolol immediate and controlled release before and after RYGB are shown along with the segmental fraction of dose absorbed along the small intestine.

In the simulated data, a very small reduction of 3% in oral bioavailability (F_{oral}) following RYGB was predicted (see Table 23).

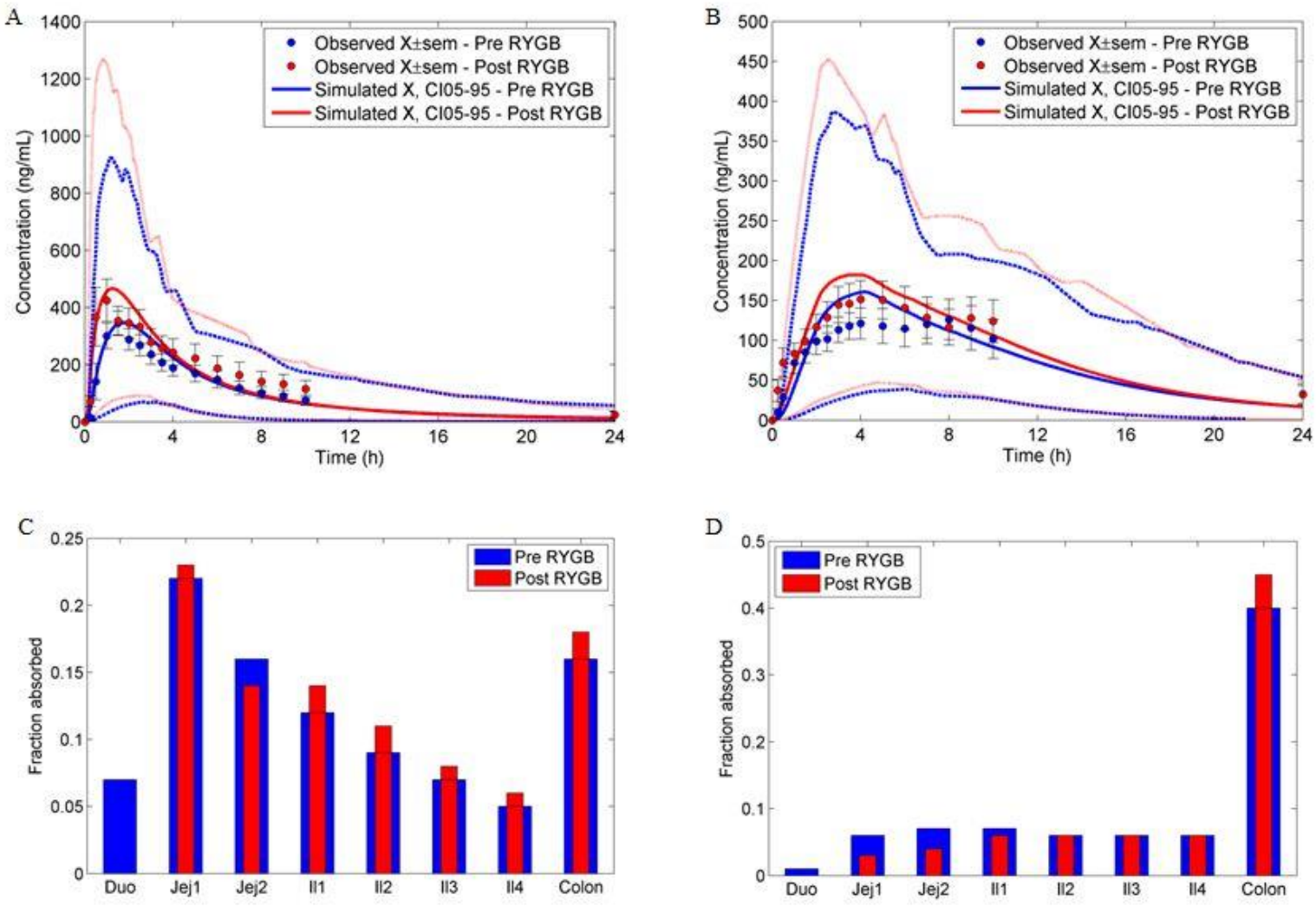


Figure 12: Mean plasma concentration-time profiles from the *in vivo* pharmacokinetic study and predicted mean plasma concentration-time profiles over 24h for the immediate release (A) and controlled release formulation (B) and predicted mean of segmental fraction of dose absorbed along the intestine for immediate release (C) and controlled release formulation (D)

Table 23: Predicted results for the bioavailability of metoprolol from an immediate release (IR) and a controlled release (CR) formulation

	F_A	F_G	F_H	F_{oral}
Metoprolol IR preoperative	0.81	0.97	0.48	0.38
Metoprolol IR postoperative	0.79	0.97	0.48	0.37
Ratio post- vs pre-RYGB	0.98	1.00	1.00	0.97
Metoprolol CR preoperative	0.81	0.97	0.48	0.38
Metoprolol CR postoperative	0.78	0.98	0.49	0.37
Ratio post- vs pre-RYGB	0.96	1.01	0.97	0.97

F_A, fraction of drug absorbed; $F_{G,}$ fraction of drug escaped gut metabolism;
F_H, fraction escaped first pass metabolism; F_{oral}, oral bioavailability

During the pharmacokinetic study, pharmacodynamic parameters were also monitored (Figure 13). Before administration of the metoprolol immediate release formulation, baseline systolic blood pressure (p=0.02) and heart rate (p<0.01) were significantly lower after RYGB; before administration of metoprolol controlled release, baseline systolic blood pressure (p=0.01) was significantly lower after RYGB. After administration of metoprolol immediate release, there was a significant interaction between time point after administration and moment of the experiment (before or after surgery) for heart rate (p=0.029) and systolic blood pressure (p<0.001), but not for diastolic blood pressure. After administration of the controlled release formulation, there were no significant interaction effects regarding the pharmacodynamic parameters.

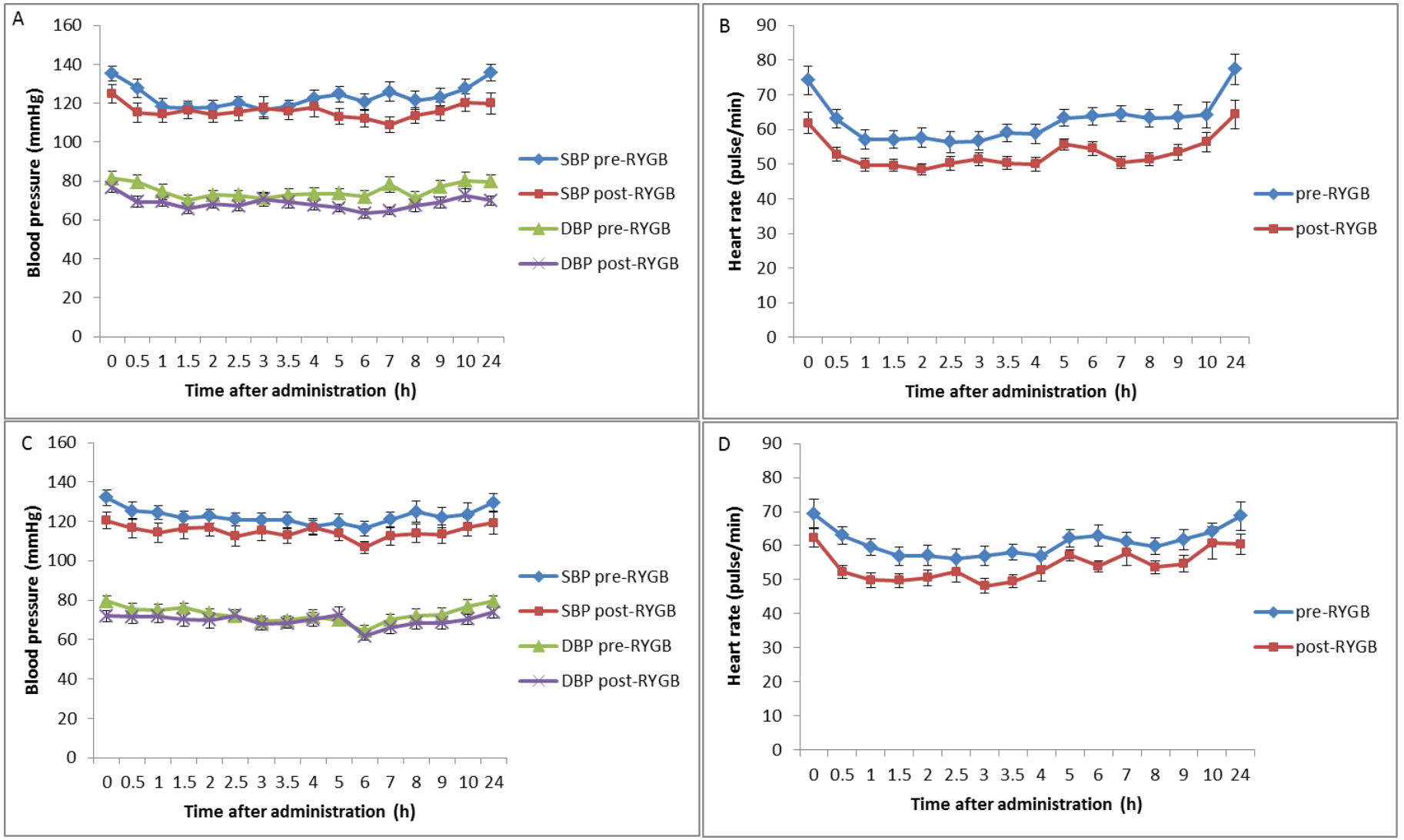


Figure 13: Pharmacodynamic parameters: systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate at the different time points after administration of the immediate formulation (A and B) and controlled release formulation (C and D), shown as mean concentration±SD

7.4 Discussion

As the knowledge regarding the impact of gastric bypass on drug disposition is very limited, this study aimed to investigate the disposition of BCS class I compound metoprolol from an immediate and controlled release formulation before and after RYGB, which would also serve as further validation of a previously developed RYGB PBPK absorption model. No significant differences were observed in the pharmacokinetic parameters of disposition of both formulations.

Metoprolol (pK_A = 9.18) is known to cross the intestinal mucosa through passive diffusion [166]. As this compound has a high solubility and high permeability [165;177], its absorption is not expected to be altered significantly after RYGB, which was indeed confirmed in this study, despite the observed tendency towards a higher oral drug exposure.

Besides its high solubility (> 700 mg metoprolol tartrate/mL in water at 37°C) and high permeability, the absorption of metoprolol by the gastrointestinal tract is rapid and complete; no site dependent absorption occurs over a large part of the intestine [177;178]. However, in view of the reduced length of the gastrointestinal tract after RYGB, the absorption may be decreased. Additionally, surgery associated weight loss might result in a reduced distribution volume, which therefore may compensate for a possible reduction in oral absorption postoperatively. In a previous study in obese patients, the apparent distribution volume for metoprolol was shown to be higher in obese patients compared to non-obese patients with a lower peak concentration [179]. This can also contribute to the tendency towards an increased oral exposure post-RYGB, since the BMI and fat percentage is decreased six months post-RYGB compared to baseline.

Furthermore, metoprolol undergoes metabolism in the liver by CYP2D6 and to a small extent by CYP3A4, resulting in the formation of metabolites (O-desmethylmetoprolol and α-hydroxymetoprolol) without a significant beta-blocking effect [180]. As no significant changes were observed in the pharmacokinetic parameters of disposition and the half-life of metoprolol before and

after surgery, CYP2D6-mediated metabolism is probably the same before and after RYGB as metoprolol is a validated probe drug for CYP2D6 activity [181].

Also for the controlled release formulation, no significant differences in the disposition of metoprolol were observed. Both formulations contained the same salt, metoprolol tartrate, and we could therefore expect these two formulations to display the same solubility properties. Previous studies have shown that the extent of absorption of metoprolol is comparable along the gastrointestinal tract [182;183]. This may explain the absence of an effect on $AUC_{0\text{-}24h}$ for the controlled release formulation before and after RYGB as the absorption in more distal parts of the intestine can compensate for the bypassed proximal segment of the small intestine. Furthermore, for a controlled release formulation based on a matrix system, as is the case here, the intestinal transit time becomes an important factor in limiting absorption. In only a few studies the intestinal transit time after RYGB has been investigated. Dirksen et al. have shown that the small intestinal transit time after a meal was slower in patients more than one year post-RYGB than in control subjects, while the colonic transit rate did not differ between the groups [65]. These observations do not entirely correspond with the findings of Morinigo et al., who have shown that the oro-caecal transit time, which includes pouch emptying and small intestinal transit, was shorter in RYGB-patients. Other studies have already shown that the gastric emptying for liquids is accelerated after RYGB [18;62;64]. Carswell et al. also reported on the oro-caecal transit time using sulphasalazine; in this study, RYGB had no impact on the oro-caecal transit time [21]. Overall, these studies indicate that the transit time before and after RYGB is probably comparable, which contributes to the similar disposition of metoprolol after administration of the controlled release formulation. Although, the oral exposure after administration of the controlled release formulation had also the tendency to be increased; this might be explained by the characteristics of the compound, as discussed for the immediate release formulation.

Because it is impossible to test all the drugs on the market in clinical trials in specific patient populations, PBPK modelling may be considered a complimentary approach in that it may provide potential insights as to what factors are mainly responsible for observed differences in drug exposure between populations. During the last few years, PBPK modelling has indeed seen an expanding area of applications, including that of post bariatric surgery patients. A pharmacokinetic model was created for the different types of bariatric surgery, including RYGB, by Darwich et al. [60]. The observed data of metoprolol immediate and controlled release were compared to matched simulations utilising the PBPK RYGB model. The trends observed in the clinical studies were comparable to the predictions made using the PBPK modelling and simulation approach. However, an overprediction occurred in the first part of the concentration-time profiles, especially for the controlled release formulation which could probably be attributed to the lack of well-established *in-vitro in-vivo* correlation methods. Despite this minor overprediction, the observed data were well within the 95% prediction intervals. According to simulations, the trend of an increased oral exposure was mainly due to weight loss as the oral bioavailability remained almost constant before and after surgery. For the observed data, weight loss could contribute to the tendency of the increased exposure of metoprolol post-RYGB.

Based on the current results one could conclude that the PBPK modelling and simulation provides a good platform for reasoning around what factors will be the most significant in determining the disposition of metoprolol following RYGB.

As already mentioned, metoprolol is metabolized by CYP2D6, which has a genetic polymorphism; a different CYP2D6 genotype and metabolizer phenotype may thus influence the pharmacokinetics of metoprolol [168;184]. Therefore, it may be advantageous to determine the genotype of the volunteers, which was not performed in this study. However, the fact that we followed the same group before and after the operation (i.e. the genotype in both groups was the same) rules out the absolute necessity of genotyping.

It also has to be kept in mind that the expression of cytochrome P450 enzymes is the highest in duodenum and jejunum and decreases towards to more distal sites of the small intestine [69]; bypassing parts of the proximal small intestine with a high abundance of CYP enzymes may therefore lead to a different effect on the bioavailability of drugs metabolized by CYP enzymes, depending on the genotype. Bypassing first-pass metabolism in the proximal small intestine may also contribute to the tendency of an increased exposure of metoprolol after RYGB as it results in a decreased presystemic biotransformation. A similar effect has been described by Skottheim et al. [76] concerning the exposure of atorvastatin following gastric bypass surgery.

In this study, we also explored the influence of both formulations on the pharmacodynamic parameters; the blood pressure and heart rate at baseline were lower after the operation. This can be explained by the improvement of cardiovascular parameters after RYGB [185].

Overall, the strength of this study lies in the fact that it was performed in the same patient group before and after the operation, and that the same type of surgery was performed by the same surgeon. This design helps to minimize inter-individual differences in metoprolol exposure before and after surgery, which is important as there are several factors contributing to the inter-individual variability in the pharmacokinetics of metoprolol (such as age, first-pass metabolism and intestinal absorption) [166].

In the future, more challenging drugs will be studied, including low solubility compounds for which solubilisation depends on intraluminal bile salt concentrations or on the residence time in the acidic environment of the stomach.

7.5 Conclusions

The oral exposure of metoprolol immediate release and controlled release formulation was not significantly different before compared to after RYGB, although a tendency towards higher exposure existed following surgery, which could be explained by weight loss and a reduced presystemic

biotransformation in the proximal GI-tract. The PBPK modelling and simulation predicted values were similar to the observed data, confirming its validity in daily clinical practice.

CHAPTER 8: DRUG DISPOSITION BEFORE AND AFTER GASTRIC BYPASS: FENOFIBRATE AND POSACONAZOLE

This chapter is based on:

Gesquiere, I., Hens, B., Van der Schueren, B., Mols, R., de Hoon, J., Lannoo, M, Matthys, C., Foulon, V., Augustijns, P. (2015)

Drug disposition before and after gastric bypass: fenofibrate and posaconazole

[Manuscript under review – Clinical Pharmacology and Therapeutics]

8 DRUG DISPOSITION BEFORE AND AFTER GASTRIC BYPASS: FENOFIBRATE AND POSACONAZOLE

Background: Roux-en-Y gastric bypass alters the anatomical structure of the GI-tract, which can result in alterations in drug disposition. The objective of this study was to evaluate oral drug disposition of two compounds belonging to the Biopharmaceutical Classification System Class II, fenofibrate and posaconazole, before and after Roux-en-Y gastric bypass (RYGB) surgery.

Methods: A single-dose pharmacokinetic study with 67 mg of fenofibrate (Lipanthyl®; n=12) and 400 mg of posaconazole (Noxafil®; n=11) was performed before and after RYGB in the same individuals. After oral administration, blood samples were collected up to 48 h after administration. Plasma concentrations were determined by HPLC in order to calculate $AUC_{0\text{-}48h}$, C_{max} and T_{max}.

Results: After administration of fenofibrate, no statistically significant differences in $AUC_{0\text{-}48h}$, C_{max} and T_{max} between pre- and post-operative setting were observed. For posaconazole, the $AUC_{0\text{-}48h}$ was 3.11±0.78 µg/mL*h before RYGB and decreased significantly to 1.81±0.20 µg/mL*h (p=0.03) after RYGB. Furthermore, a significant decrease in C_{max} was observed following RYGB (p=0.03). The decreased exposure of posaconazole could be explained by the increased gastric pH and accelerated gastric emptying of fluids post-RYGB. No significant difference for T_{max} was observed.

Conclusions: The disposition of fenofibrate was not significantly altered after RYGB, whereas the oral disposition of posaconazole was significantly decreased following RYGB.

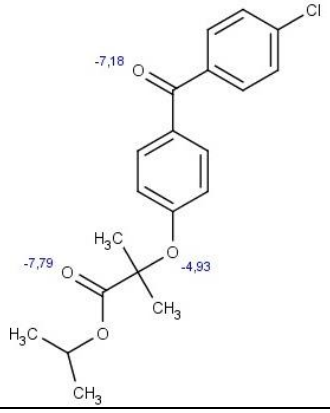
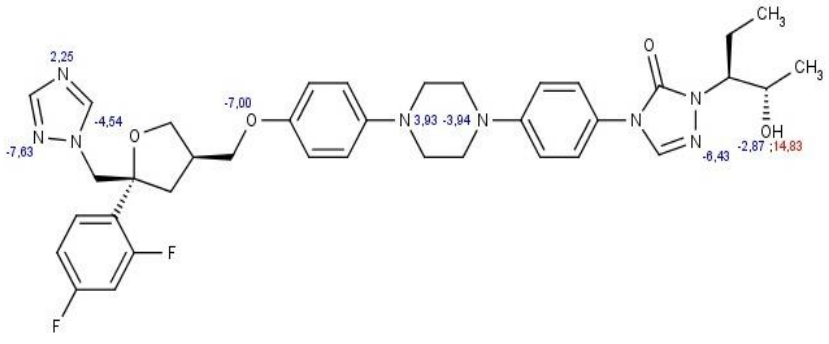
8.1 Introduction

Over the last decades, the prevalence of obesity has increased dramatically [1]. This has led to an increased demand for bariatric surgery, especially Roux-en-Y gastric bypass (RYGB), which is the only available treatment leading to major and sustainable weight reduction in morbid obese patients (BMI ≥ 40 kg/m² or BMI ≥ 35 kg/m² with obesity-related diseases) [141]. RYGB results in an altered anatomical structure and physiology of the gastrointestinal tract by reducing the gastric capacity and bypassing the duodenum and the proximal jejunum [58;59]. These changes can alter the pharmacokinetics of a given drug. However, the extent to which absorption is altered for a specific drug or class of drugs remains unknown. The few studies that have been conducted illustrate that a trend in oral drug exposure before to after RYGB is not easy to predict. Oral exposure can be reduced following RYGB (as reported for azithromycin [164]); it can remain unaltered (as reported for levothyroxine [79]); or can be increased (as for metformin [72]). A systematic approach to study the influence of RYGB on oral drug exposure is lacking as previously conducted studies vary in design and are poorly standardized based on how surgery is conducted, which makes the results difficult to compare. We have already performed a pharmacokinetic study with metoprolol in patients before and after RYGB and the same design is used in the current pharmacokinetic study, which allows comparison of the results.

As mentioned above, a lot of anatomical and physiological changes are associated with a RYGB. In patients with a RYGB, the gastric remnant and bypassed biliary limb is reconnected to the intestine, 75 to 150 cm distal to the anastomosis between the gastric pouch and the distal part of the jejunum [12]. This results in a delayed inlet of bile acids in the intestine. Furthermore, a RYGB is associated with the formation of a small gastric pouch, resulting in an increased gastric pH; an increased gastric pH may also be due to the widespread use of antacid medication following surgery. The increased gastric pH has an influence on drug dissolution and solubility of ionizable compounds, and subsequently on their absorption [31;32]. Regarding these changes, we have chosen drugs substances based on their absorption characteristics. In this study, we have investigated the influence of RYGB

on the disposition of fenofibrate and posaconazole. Both compounds belong to the Biopharmaceutical Classification System (BCS) class II. The BCS classifies drug substances depending on their solubility and permeability, and class II drugs are characterized by a high permeability, but a low solubility [165]. Fenofibrate (neutral) has been selected as a test compound because it has been demonstrated that solubility highly depends on bile salt concentrations. In view of a delayed contact with bile salts after surgery, a decrease in absorption was expected. Posaconazole (weak base) has been selected as a model compound in view of the fact that a relation has been demonstrated between the residence time in the acidic environment of the stomach and systemic exposure. In view of a reduced acid production and a shorter residence time in the stomach after RYGB, a lower systemic exposure was expected. The structure and physicochemical properties of fenofibrate and posaconazole are shown in Table 24.

Table 24: Structure and physicochemical properties of fenofibrate and posaconazole

	Structure with pKa values	**Molecular weight (g/mol)**	**LogP**
Fenofibrate		360.8	5.28
Posaconazole		700.8	5.41

Fenofibrate is a lipid lowering agent and is in Belgium available as a micronized formulation (Lipanthyl® capsule) and a nanonized formulation (Lipanthylnano® tablet). In this study, we have

chosen for the micronized tablet as our focus was to analyze the drug substance and not the specialized formulation. Posaconazole is a broad-spectrum anti-fungal drug for the treatment of invasive fungal infections and is in Belgium available as a suspension (Noxafil®) [186].

8.2 Methods

8.2.1 Selection of patients

For this study, 24 obese patients with a planned RYGB surgery at the University Hospitals Leuven, Belgium were recruited; 12 patients for the study with fenofibrate and 12 patients for the study with posaconazole. Patients who had previously undergone bariatric surgery or who had renal and hepatic impairment were not included in the study. Pregnant and breastfeeding women were also not included. RYGB surgery was performed in all recruited patients by the same surgeon and according to the same procedure. In brief, the jejunum was divided 30 cm from the ligament of Treitz and anastomosed to a 30 mL proximal gastric pouch. The jejunum was reanastomosed 120 cm distally to the gastrojejunostomy. All mesenteric defects were closed. The study was approved by the Medical Ethics Committee of the University Hospitals Leuven (ML8433) and is saved in the European Clinical Trials database (EudraCT) with reference number 2012-001244-22. All patients gave written informed consent.

8.2.2 Study design and procedure

In one group (12 patients; 11 Caucasian and 1 African), a single-dose pharmacokinetic study with 67 mg of fenofibrate (Lipanthyl®) was performed before and six to nine months after RYGB (on average 6.9 months (SD 1.0); further referred to as six months after RYGB). In another group of 12 patients (10 Caucasian, 1 African and 1 South-American), a single-dose pharmacokinetic study with 400 mg of posaconazole (10 mL of Noxafil®, an oral suspension containing 40 mg/mL) was performed before and six to nine months after RYGB (on average 6.7 months (SD 0.7); further referred to as six months after RYGB). Because lung cancer was diagnosed in a patient post-RYGB, there was a drop-out of one participant in the posaconazole study.

The extent of disposition of fenofibrate and posaconazole was estimated by the determination of the area under the curve ($AUC_{0\text{-}48h}$), the peak plasma concentration (C_{max}) and the time to reach peak concentration (T_{max}) of fenofibric acid and posaconazole, respectively.

Following an overnight fast of at least 10 hours, subjects came to the clinical pharmacology unit of the University Hospitals Leuven. Weight and height of the subjects were measured with calibrated equipment. The weight was measured to the nearest 0.1 kg, with the subjects having an emptied bladder and wearing indoor clothing with empty pockets and without shoes. BMI (kg/m²) was calculated by dividing the weight (kg) by the square of the height (m²). The percentage of excess weight loss was calculated using the formula:

$$\%EWL = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight} - \text{ideal body weight}} \quad [102]$$

A Dual Energy X-ray Absorptiometry (DXA) was performed to measure the amount of body fat mass [169].

After the insertion of an intravenous catheter, one group of the subjects ingested 67 mg of fenofibrate (Lipanthyl®) with 150 mL of water, the other group ingested 10 mL of Noxafil®, corresponding to 400 mg of posaconazole, with 150 mL of water. After oral administration, blood samples were collected into heparinized tubes at 15; 30; 60; 90 minutes and 2; 2.5; 3; 3.5; 4; 5; 6; 7; 8; 9; 10; 24 and 48 hours. The blood samples were centrifuged immediately after collection (1800 g, 10 min, 4°C) and plasma samples were stored at -20°C until analysis.

A standardized meal and a standardized snack were administered 4 hours and 8 hours after drug administration, respectively. Participants had to consume the entire meal. The use of water was allowed ad libitum, except for one hour before and four hours after drug administration. During the first 4 hours after administration of the drugs, the patients had to remain semi-supine in bed. After the 10h-blood sample, the subjects were discharged and had to return the two following mornings for the 24-h and 48-h blood sampling. As proton pump inhibitors (PPI), H_2-receptor antagonists and antacids could influence the absorption of drugs, the recruited patients were asked to stop these drugs during the week preceding the study. Other prescription drugs were checked to verify that

there were no pharmacokinetic interactions with the study drug. The first morning of the study, the patients were not allowed to take their medication.

8.2.3 HPLC analysis

FENOFIBRATE

After absorption of fenofibrate, it is quantitatively converted to its active metabolite, fenofibric acid [187]. Fenofibric acid was determined in plasma to assess the oral exposure from fenofibrate. Before HPLC-UV analysis, fenofibric acid (active metabolite) was extracted from plasma samples. 100 µL of a stock solution of the internal standard solution carbamazepine (20 µM in 1 M HCl) was added to 500 µL of plasma. Subsequently, 400 µL of HCl (1 M) was added and vortexed (± 10 sec) in order to precipitate plasma proteins. To extract fenofibric acid and carbamazepine, 6 mL of dichloromethane was added and samples were shaken for one minute. After centrifugation (2880 g, 15 min, 4°C), the water layer was discarded and the organic layer was evaporated under a stream of air until dryness. The residue was dissolved in 1 mL of methanol. Following evaporation, 200 µL of mobile phase was added to the residue and injected into the HPLC system. Carbamazepine and fenofibric acid were detected at a wavelength of 287 nm (Waters 2487 UV Detector). A retention time of 4.5 and 8 min were generated with a flow rate of 1 mL/min for carbamazepine and fenofibric acid, respectively. Running conditions started with acetonitrile:25 mM acetic acid buffer pH 3.5 (50:50 v/v). Acetonitrile concentrations increased up to 60% over 3 min. Following elution of fenofibric acid, the column was rinsed during 2 min with acetonitrile:water (90:10 v/v), followed by 1 min with water:25 mM acetic acid buffer pH 3.5 (75:25 v/v) and subsequently re-equilibrated with the starting conditions (acetonitrile:25 mM acetic acid buffer pH 3.5 (50:50 v/v)) for 2 min.

The calibration curve was based on a stock solution of fenofibric acid in acetonitrile. Blank plasma samples were spiked and treated the same way as the samples. Linearity was observed between 158 µM and 0.31 µM. Method validation resulted in accuracy and precision errors of less than 5% and 8%, respectively, for a concentration of 9.8 µM. Quality control samples (9.8 µM) were included on the days of analysis and resulted in a relative standard deviation of less than 5%.

POSACONAZOLE

Analysis was performed by extracting posaconazole from plasma samples as described by Walravens et al. [188]. Concentrations of posaconazole were determined using HPLC/fluorescence analysis. Briefly, 100 µL of internal standard solution (2.5 µM itraconazole in 0.2 N HCl) was added to 1000 µL of plasma. Subsequently, the sample was alkalized with 500 µL of 2 N NaOH. After addition of 4 mL diethylether, samples were vortexed for 30 seconds and directly centrifuged (2880 g, 5 min, 4°C). Finally, the organic layer was transferred to a clean glass tube and evaporated to dryness under a gentle stream of air. A volume of 300 µL of mobile phase (methanol: 20 mM acetic acid buffer pH 3.3 (76:24 v/v)) was added to the remaining residue. After centrifugation (2880 g, 5 min, 4°C), 50 µL of the supernatant was injected into the Hitachi Elite LaChrom HPLC system (VWR International) and analyzed by the Hitachi Elite LaChrom L-2480 fluorescence detector (excitation wavelength 240 nm, emission wavelength 385 nm). A gradient run of 19 min was performed in order to obtain a retention time of 7.9 and 12.1 min on the Novapak C-18 column for posaconazole and itraconazole, respectively. Gradient elution at a constant flow rate of 1 mL/min was performed as follows: MeOH: 20 mM acetic acid buffer pH 3.3 (76:24) for 2 min followed by MeOH: 20 mM acetic acid buffer pH 3.3 (81:19) for 7 min; followed by a rinsing step with 100% MeOH for 3 min; then re-equilibration for 5 min with MeOH: 20 mM acetic acid buffer pH 3.3 (76:24) before the next injection. A calibration curve was made based on stock solutions of posaconazole and itraconazole in DMSO. Linearity was observed between 2000 nM and 7.8 nM. Quality control samples of 500 and 50 nM, which were analyzed together with the plasma samples, resulted in an accuracy error of less than 10%.

8.2.4 Data analysis

The $AUC_{0\text{-}48h}$ of the concentration-versus-time curves was determined using the linear trapezoidal rule. All results are presented as mean±SEM, unless otherwise mentioned. To evaluate the effect of RYGB on the pharmacokinetic parameters of fenofibrate and posaconazole, $AUC_{0\text{-}48h}$, C_{max} and T_{max} obtained before and after surgery were compared. The paired data were analyzed with SPSS Statistics 22, performing a paired *t*-test as the assumption for normal distribution of the data was

accepted (Shapiro-Wilk test). The $AUC_{0\text{-}48h}$ and C_{max} of posaconazole were transformed with the logarithm function to achieve normality. As no normality was achieved for T_{max}, a Wilcoxon signed-rank test was performed. Multiple linear regression analysis was performed to control for correlation between the difference of $AUC_{0\text{-}48h}$ and gender, age, difference in BMI, fat percentage measured by DXA and %EWL. Statistical significance was set at $p<0.05$.

8.3 Results

8.3.1 Fenofibrate

For fenofibrate, we recruited 12 patients (7 female, 5 male) with a mean age of 43.3±4.2 years. The characteristics of these patients are summarized in Table 25. Weight, BMI, %EWL and fat mass percentage were significantly decreased post-RYGB.

Table 25: Characteristics of the participants in the fenofibrate pharmacokinetic study (n=12), shown as mean±SEM

	Before RYGB	**After RYGB**
Weight (kg)	117.9±5.9	83.7±4.3*
BMI (kg/m²)	40.4±1.0	28.8±1.0*
% weight loss	0	63.3±4.0*
Fat percentage (%)	42.5±1.9	31.0±2.7*

*$p<0.05$

The observed concentration-time profiles are shown in Figure 14. No significant differences were observed for the $AUC_{0\text{-}48h}$, C_{max} and T_{max} of fenofibrate before and after RYGB (see Table 26).

The half-life of fenofibrate was 27.4±3.6 h before RYGB and 28.3±2.3 h post-RYGB; no significant difference was observed. By comparing the individual $AUC_{0\text{-}48h}$ postoperative with preoperative, two patients had more than 25% decrease in $AUC_{0\text{-}48h}$ and 4 more than 25% increase in $AUC_{0\text{-}48h}$. For the others, the $AUC_{0\text{-}48h}$ was comparable before and after surgery. No correlation with other variables was identified.

Table 26: Pharmacokinetic results for fenofibrate before and after surgery, shown as mean±SEM

	$AUC_{0\text{-}48h}$ (µg/mL*h)	C_{max} (µg/mL)	T_{max} (h)
Fenofibrate preoperative	31.3±4.33	1.37±0.23	4.5±0.5
Fenofibrate postoperative	33.3±3.73	1.57±0.25	5.1±1.8
Post- vs pre-RYGB	Ratio	Ratio	Difference
	1.06	1.14	0.6
Significance level	*p*=0.54	*p*=0.36	*p*=0.31

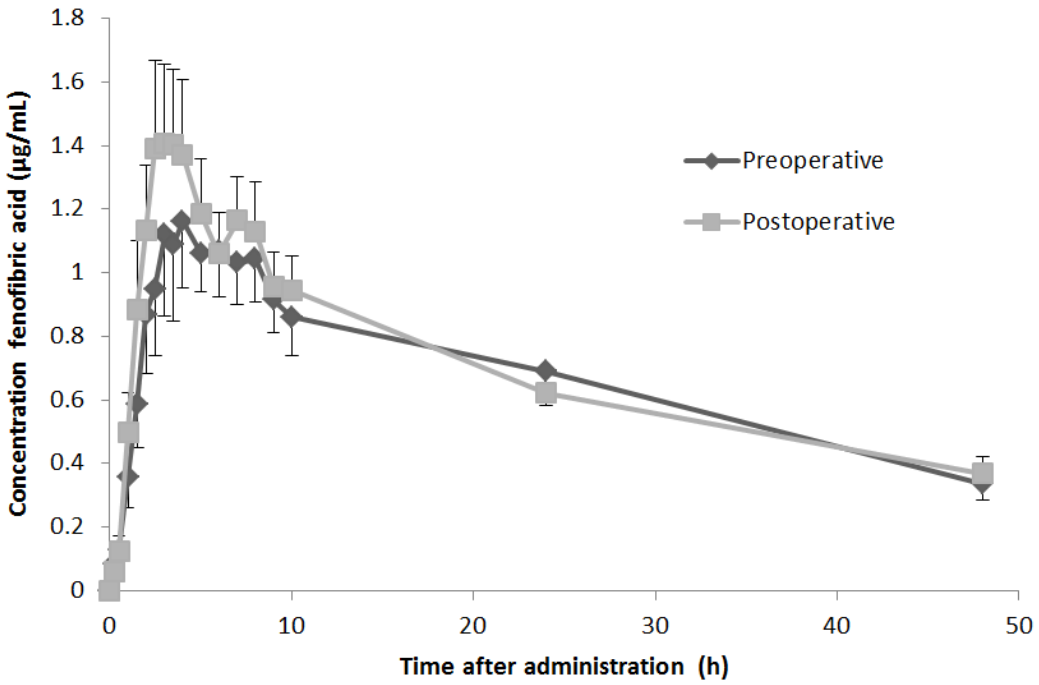


Figure 14: Observed plasma concentration-time profiles of fenofibrate over 48 h after oral administration, shown as mean concentration±SEM

8.3.2 Posaconazole

The results from the pharmacokinetic study with posaconazole are from 11 patients (7 female, 4 male) with a mean age of 37.4±3.3 years. The characteristics of the participants are shown in Table 27. Weight, BMI, %EWL and fat mass percentage were significantly decreased post-RYGB.

Table 27: Characteristics of the participants in the posaconazole pharmacokinetic study (n=11), shown as mean±SEM

	Before RYGB	**After RYGB**
Weight (kg)	122.8±5.6	87.2±4.2*
BMI (kg/m²)	40.8±1.7	29.0±1.3*
% weight loss	0	64.8±6.2*
Fat percentage (%)	41.9±2.3	31.6±2.6*

*$p<0.05$

The observed pharmacokinetic results of posaconazole are summarised in Table 28 and the observed concentration-time profiles are shown in Figure 15.

A statistically significant difference in the observed $AUC_{0\text{-}48h}$ after oral administration of posaconazole was shown (paired *t*-test: $p=0.03$). The $AUC_{0\text{-}48h}$ post-RYGB was 58% of the $AUC_{0\text{-}48h}$ before RYGB. C_{max} was also significantly decreased after surgery: C_{max} post-RYGB was 47% of C_{max} preoperative (paired *t*-test: $p=0.03$). The time to reach maximum plasma concentration was not statistically significant different (Wilcoxon: $p=0.33$).

The half-life of posaconazole was increased post-RYGB, but the difference was not statistically significant (54.3±4.5 h pre-RYGB to 79.3±11.9 h post-RYGB). No correlation with other variables was identified.

Table 28: Pharmacokinetic results for posaconazole before and after surgery, shown as mean±SEM

	$AUC_{0\text{-}48h}$ (µg/mL*h)	**C_{max} (µg/mL)**	**T_{max} (h)**
Posaconazole preoperative	3.11±0.78	0.12±0.04	7.7±4.1
Posaconazole postoperative	1.81±0.20	0.06±0.01	6.4±1.8
Post- vs pre-RYGB	Ratio	Ratio	Difference
Significance level	0.58 $p=0.03$	0.47 $p=0.03$	-1.3 $p=0.33$

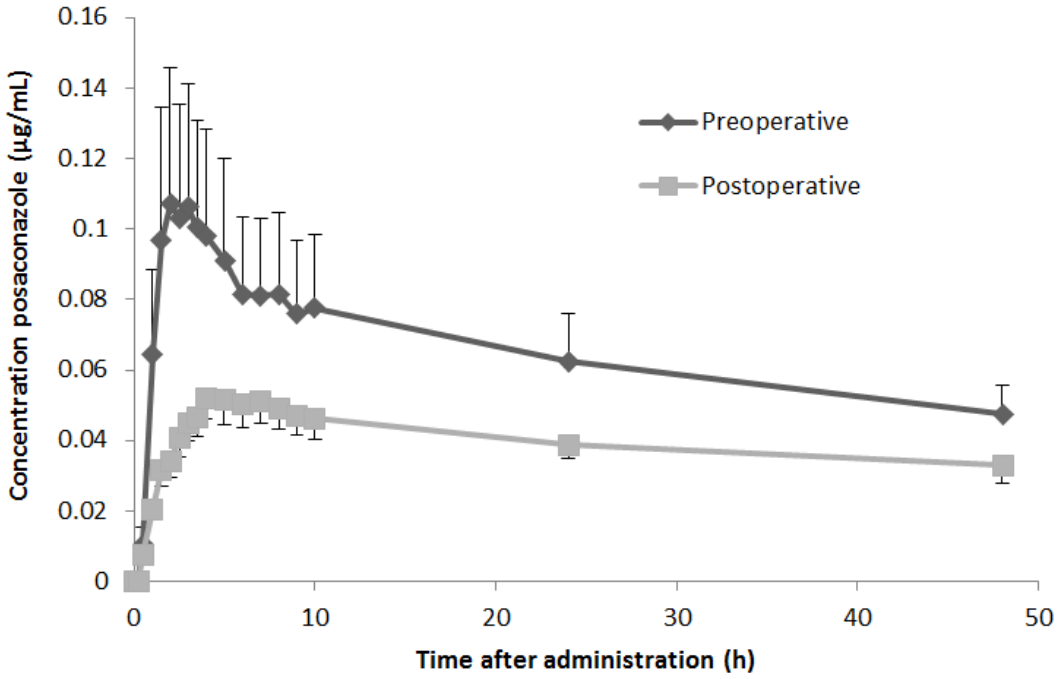


Figure 15: Observed plasma concentration-time profiles of posaconazole over 48 h after oral administration, shown as mean concentration±SEM

8.4 Discussion

In this study, we investigated the pharmacokinetic parameters of two lipophilic drugs, being a neutral compound (fenofibrate) and a weak base (posaconazole) in patients before and after RYGB. We showed that the mean pharmacokinetic parameters of the neutral compound, fenofibrate were unaltered after RYGB, whereas for the weak base, posaconazole, the oral exposure was significantly decreased after RYGB.

Both fenofibrate and posaconazole, are lipophilic drugs belonging to BCS class II (low solubility, high permeability). The solubility of fenofibrate highly depends on bile salt/phospholipid concentrations. These ingredients are responsible for the formation of micelles, which increase the solubility of lipophilic drugs. A RYGB changes the anatomical structure of the gastrointestinal tract, resulting in a delayed inlet of bile acids [12]. This delay could explain the delayed exposure of fenofibrate to bile acids, which can result in a reduced exposure after oral administration. However, the mean oral exposure of fenofibrate was not significantly different before and after RYGB. This could be explained by a compensation mechanism after RYGB. Patti et al. [66] have shown that the fasting total serum

bile acids concentration is twice as high in patients 2 to 4 years after RYGB, compared to overweight or obese individuals without bariatric surgery. This has been confirmed in patients 4 days post-RYGB and in patients 1 year post-RYGB [67]. This increase might be associated with an increased secretion of bile acids in the small intestine, compensating for the delayed inlet of bile acids post-RYGB and resulting in no significant differences in pharmacokinetic parameters of oral fenofibrate disposition after RYGB. However, no information about the concentration of bile acids in the intestine after RYGB is available. Interestingly, there was no significant delay in time to reach peak plasma concentration of fenofibrate. It is possible to hypothesize that the interval between administration of the drug and contact between bile acids and drugs is comparable before and after surgery, despite the delayed inlet of bile acids. This might be explained by the fact that the delayed inlet of bile acids might be compensated by the accelerated gastric emptying of fluids after RYGB and thus be similar; accelerated gastric emptying has already been shown in previous studies using different methods [18;62-65].

It is worth mentioning that large inter-individual differences were observed: some patients had a decreased oral exposure of fenofibrate, others an increased; for most patients the oral exposure pre- and postoperative was the same. This might reflect large inter- and intra-individual differences in the amount of bile acid secretion.

Posaconazole is a lipophilic, weak base and we have shown that the $AUC_{0\text{-}48h}$ and C_{max} of this compound was significantly decreased post-RYGB [189]. Dissolved posaconazole might be absorbed quickly as it has a high permeability. However, it has a low solubility and the intraluminal pH and the residence time in the stomach have an important role in the intestinal absorption of posaconazole: a previous study has shown a relation between the oral exposure of posaconazole and the residence time in the acidic environment of the stomach [188].

In RYGB patients, a small gastric pouch, which is separated from the native stomach, is created. Previous studies have shown that the gastric acid secretion after gastric bypass is negligible as the

majority of the parietal cells (i.e. acid-producing cells) are bypassed [31;32]. This results in an elevated gastric pH, which impacts the solubility of drugs, including posaconazole as a weak base. In a previous study, it has been shown that the oral exposure and C_{max} of posaconazole was decreased if the gastric pH was increased by the intake of a PPI (esomeprazole), and was increased when posaconazole was administrated with an acidic beverage [190]. Walravens et al. have confirmed that an increased gastric pH is associated with a reduced absorption of posaconazole, while a longer residence time in the stomach increased absorption [188]. After RYGB, both factors (gastric pH and residence time in the stomach) are changed. Regarding gastric emptying, it has been shown by different methods that the gastric emptying for liquids is accelerated after RYGB [18;62-65]. A faster gastric emptying is associated with a shorter residence time in the stomach and subsequently a shorter time period for posaconazole to dissolve, eventually resulting in a reduced $AUC_{0\text{-}48h}$ and C_{max}, and especially a reduced absorption.

These observations may have significant clinical implications. The results from our study can be an explanation for a case in which ineffectiveness of posaconazole in a RYGB-patient was reported [85]. The patient was treated with posaconazole during 10 days and the levels were well below the minimal inhibitory concentration, despite strict adherence and no co-medication that could interfere with the absorption of posaconazole. A switch to oral isavuconazole was necessary in this case [85]. This highlights the importance of pharmacokinetic studies in this patient population in order to avoid potentially dangerous under-dosing. Therapeutic drug monitoring for posaconazole after RYGB should be considered in order to ensure reaching minimal inhibitory concentrations.

Furthermore, we need to take into account that drug absorption might have been impaired before RYGB, as obesity can also have an influence on drug disposition [191]. However, to our knowledge, data about pharmacokinetics of fenofibrate and posaconazole in obese individuals are not available. From a theoretical point of view, the elimination of fenofibrate and posaconazole in obese individuals might be increased. Drug elimination of fenofibric acid and posaconazole is mainly

mediated by glucuronide conjugation [192]. In a previous study in obese individuals, an increase in glucuronidation was shown [193]. However, no significant difference in half-life of fenofibrate was observed in our study. Glucuronide conjugation is potentially comparable before and after RYGB. For posaconazole, we have observed a longer half-life post-RYGB, which might be explained by the decreased intraluminal dissolution/solubility of posaconazole during which absorption could take place, which might be associated with a longer time-window for absorption. Furthermore, fenofibric acid has a high plasma protein binding (more than 95% binds to serum albumin) [192]. Some previous studies have shown that obesity has no influence on binding of drugs to albumin [194-196].

Moreover, obesity is associated with a lot of changes regarding body composition, which can have an influence on the volume of distribution (V_D), especially for lipophilic compounds. In general, lipophilic compounds will have an increase in V_D in obese versus normal weight individuals; however there are exceptions [197;198]. Both fenofibrate and posaconazole are lipophilic drugs and may be associated with an increased V_D before RYGB as obesity is associated with an excessive fat mass accumulation. Especially posaconazole has a remarkable distribution in the body as 40-fold higher tissue concentrations compared to serum concentrations are described [199]. We have shown that the fat mass percentage after surgery was significantly decreased, which might have resulted in a decreased V_D post-RYGB. These changes might be reflected in lower plasma concentrations before surgery.

To increase the solubility of posaconazole, co-administration of an acidic carbonated beverage, such as Coca-Cola®, can help [188]. But in patients with RYGB, the intake of this kind of drinks is not recommended as it can result in gastric problems and dumping syndrome [109]. Furthermore, the intake of fenofibrate and posaconazole with high-fat food could increase intraluminal solubility by an increased bile acid flow [199]. However, in this study we preferred to administer the drugs in a fasted state, which had two reasons. First, results regarding gastric emptying of solids after RYGB are contradictory [63;65]. This implies that if the drug was administered during a fed state, more inter-individual differences would be observed. Furthermore, we wanted to use the same design as in our

previously performed pharmacokinetic study with metoprolol, in order to allow comparison of the different analyzed drugs (Chapter 7).

Recently, a delayed-release tablet formulation containing 100 mg of posaconazole has been developed using hot-melt extrusion technique, in which posaconazole is dispersed in hypromellose acetate succinate (HPMCAS), that is pH-sensitive [199;200]. The tablet has the advantage that it results in a higher exposure to the drug, and less inter-individual differences. Moreover, the exposure from the tablet was similar when drugs affecting gastric pH and motility were administered at the same time [199]. Maybe the increased gastric pH post-RYGB would have less influence on posaconazole exposure from the tablet formulation than from the oral suspension. In a future study, it would be interesting to test the tablet formulation of posaconazole in this population group in order to have a better idea of the influence of RYGB on different types of formulations of the same drug, as we did already for metoprolol (immediate and controlled release formulation).

In the current study, we only followed plasma concentrations of fenofibrate and posaconazole. It would be interesting, however, to monitor both plasma and gastrointestinal concentrations simultaneously. This would allow combining data on gastrointestinal behavior of fenofibrate and posaconazole with data on their intestinal absorption. This could be realized by collection of gastrointestinal fluids through catheters positioned into the stomach and the small intestine [201]. Furthermore, measuring the gastric pH at time of administration could be interesting.

Overall, the strength of our pharmacokinetic study is the design as it has been performed in the same patient group before and after the operation, ensuring no inter-individual differences between both groups. Furthermore, the patients underwent the same type of surgery, performed by the same surgeon.

8.5 Conclusions

Fenofibrate and posaconazole are both compounds of BCS class II with a high permeability and low solubility; with the difference that fenofibrate is a neutral compound and posaconazole a weak base. The pharmacokinetic parameters for the disposition of fenofibrate are not significantly different after RYGB. This is in contrast with the significantly decreased oral exposure of posaconazole after RYGB, which could be explained by the increased gastric pH and accelerated gastric emptying of fluids post-RYGB. Caution is needed when prescribing other basic drugs which are highly dependent on the acidic environment in the stomach for dissolution/solubility. The disposition of these drugs might also be decreased, resulting in under-dosing.

PART V: INFLUENCE OF RYGB ON MEDICATION COST

CHAPTER 9: MEDICATION COST IS SIGNIFICANTLY REDUCED AFTER ROUX-EN-Y GASTRIC BYPASS IN OBESE PATIENTS

This chapter is based on:

Gesquiere, I.*, Aron-Wisnewsky, J.*, Foulon, V., Haggege, S., Van der Schueren, B., Augustijns, P., Bouillot, J., Clement, K., Basdevant, A., Oppert, J., Buyse, M. (2014)
Medication cost is significantly reduced after Roux-en-Y gastric bypass in obese patients
Obesity Surgery, 24(11), 1896-1903.

(with permission from Obesity Surgery)

* These authors have equally contributed to this work.

9 MEDICATION COST IS SIGNIFICANTLY REDUCED AFTER ROUX-EN-Y GASTRIC BYPASS IN OBESE PATIENTS

Background: This study aims to determine the influence of Roux-en-Y gastric bypass (RYGB) on medication-related costs.

Methods: The study analyzed the types, dosages, and costs of drugs and medical devices prescribed before and after surgery (1, 3, 6, 12 months and yearly thereafter) in patients who underwent RYGB between June 2004 and May 2010 and had an outpatient visit between December 2009 and May 2010 at Pitié-Salpêtrière University Hospital, Paris, France.

Results: The cohort included 143 patients (78% female; mean age, 42.9 years; mean BMI, 48.6 kg/m²). Total prescription costs were significantly lower (-32%, $p<0.001$) 1 year after RYGB compared with preoperative costs. However, the cost for medications to prevent RYGB side effects (in particular nutritional deficiencies) displayed a 36-fold increase in the first month post-surgery, but then decreased progressively over time. Importantly, the cost related to the treatment of the two most frequent obesity-related diseases significantly decreased 1 year after surgery. Indeed, prescription costs for treatment of type 2 diabetes mellitus (T2DM) and obstructive sleep apnea (OSA) (namely CPAP therapy considered as the gold standard treatment) were reduced 1 year after surgery by 85 and by 63% (both $p<0.001$), respectively. We also observed a trend toward a decrease in the prescription costs of other obesity-related diseases, although it did not reach significance in our cohort.

Conclusions*:* Considering medication to treat both obesity-related diseases and prevention of secondary effects of bariatric surgery, we observed that overall postoperative medication costs were significantly reduced 1 year after surgery, especially for T2DM and OSA.

9.1 Introduction

The prevalence of obesity has increased worldwide over the last decades [156], thereby also increasing the prevalence of obesity-related diseases (such as type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension, cardiovascular diseases (CVD), but also obstructive sleep apnea (OSA), asthma, joint arthritis, and depression) [4]. Therefore, obese individuals are prone to increased consumption of drugs compared to lean individuals [5].

Medical management of obesity has proven disappointing on both the amount of weight loss and its maintenance over time [7]. Therefore, the number of patients undergoing bariatric surgery such as Roux-en-Y gastric bypass (RYGB), which is indicated for the most severe form of obesity, has increased dramatically. Most importantly, it is currently the only efficient mean to achieve major and sustainable weight reduction [4;202]. Along with a significant reduction in all-cause mortality, RYGB improves several obesity-related diseases [8-10;99;203], leading to a decreased consumption of related treatments [52]. Indeed, medication used for T2DM, hypertension and dyslipidemia decreased significantly as early as 1 year post-surgery (by 76, 51 and 59%, respectively) [53]. This reduction continued significantly 3 years post-surgery, in particular for T2DM, dyslipidemia and CVD [52]. Finally, comparing obese patients post-surgery to an obese matched control group, the SOS study, demonstrated a significant reduction in the cost of drugs for obesity-related diseases after 7 years, which remained significantly lower during 20 years of follow-up [54].

Although beneficial regarding mortality and obesity-related comorbidities, RYGB induces potential risks such as nutritional deficiencies and short-term surgical complications such as venous thrombosis, anastomosis ulceration and gallstones [56;204;205].

Both can be prevented by specific drug prescriptions such as daily vitamin and mineral substitution [55;206], hence inducing substantial costs. Few studies (if any) have yet addressed the overall treatment cost evolution after surgery, taking into account not only treatment of obesity-related diseases but also surgery side effects' prevention. As it remains unclear to what extent RYGB may be cost-saving

in terms of overall drug consumption, we aimed to examine the evolution of medication use and associated costs in RYGB patients.

9.2 Methods and procedures

9.2.1 Study design and data collection

We took advantage of an ongoing clinical research protocol (approved by the Ethics Committee of Hôtel-Dieu Hospital) including all of our bariatric surgery patients followed at the Nutrition Department of Pitié-Salpêtrière Hospital Paris, France. All subjects gave written informed consent. Only those who underwent RYGB were included. Bariatric surgery was decided in agreement with international clinical practice guidelines [207]. For the purpose of the study, patients who had undergone a previous bariatric surgery, as well as those who did not attend postoperative follow-up, were excluded.

We first retrospectively included all patients who had undergone RYGB from June 2004 to November 2009, and for whom clinical data (age, sex, BMI) were available in our database both before (baseline) and 1, 3, 6 months (M) and yearly (Y) after surgery, until 4 years post-RYGB, and who had a follow-up visit. We also prospectively included patients who came for a RYGB preoperative examination between December 2009 and May 2010. Data were collected from patient's medical records and verified with the patient at the inclusion visit. Entire obesity-related diseases history and complete list of drugs and medical devices prescribed at each examination (including Continuous Positive Airway Pressure (CPAP), considered as the gold standard treatment of OSA) [208] was retrieved. The dosing regimen for each drug was also collected.

9.2.2 Data analysis

We tabulated drugs taken by each patient at each follow-up time point, including cost per month (in Euros). The cost for each medication or medical device was obtained from the official current price list (http://www.theriaque.org and www.ameli.fr) and when appropriate, generic drug costs for the largest package available, were used. Treatments were divided into curative use (further grouped

according to the different obesity-related diseases they were used for (including CPAP treatment)), or preventive use (those to prevent surgery-related complications or potential nutritional deficiencies). The different categories of obesity-related diseases were defined upon medication use and thorough checking of the adequacy with each patient's medical history. Patients with T2DM were defined as those on oral antidiabetic therapy and/or insulin and/or GLP-1 analogue. OSA included patients on CPAP treatment. CVD comprised antihypertensive drugs and/or antiplatelet agents and included secondary CVD prevention drugs. Dyslipidemia included patients treated with statins or fibrates. Psycho- and neurological disorders comprised patients who were on antidepressant therapy and those with anti-epileptic treatment. Lastly, a category called "other" included patients using pain killers, drugs used for asthma, hyperuricemia, skin complications, urinary incontinence, or gastrointestinal problems.

We used the Anatomical Therapeutic Chemical classification system (ATC system), which categories drugs into different classes according to their therapeutic and chemical characteristics, at the first level, except for drugs used in metabolic disorders, which were classified at level 2 of the ATC system (http://www.whocc.no/). Prevention treatment at our center has previously been described [54] and includes 2 weeks before surgery the following: vitamin D (once 4x100,000 IU), vitamin B_1 (250 mg/day), vitamin B_{12} (250 µg/day) and esomeprazole (40 mg/day). Esomeprazole dosing is maintained during 6 months post-surgery to prevent anastomosis ulcerations. Enoxaparine (4,000 IU 1 inj/day) is prescribed for 3 weeks post-surgery to prevent venous thrombosis. Fifteen days post-RYGB, vitamin and mineral supplements including Azinc optimal® (2x/day), iron (2x80 mg/day), vitamin D (800 IU/day), and calcium (1,000 mg/day) are started and continued on the long term. Ursodeoxycholic acid is introduced 2 weeks post-surgery (only for patients with no previous history of cholecystectomy) and continued for 3 months to prevent gallstone formation known to be associated with major and rapid weight loss [56;204;205;207]. Besides this standard protocol, additional drugs and/or supplements to treat side effects related to surgery (vitamins, minerals and proteins, and antiemetics) can be added on an individually-tailored basis.

9.2.3 Statistical analysis

Continuous variables are presented as mean±SD. The main outcome variables were the changes in the relative cost of each comorbidity treatment, calculated both in the whole cohort or considering only those patients affected with the comorbidity in question. Changes in (1) the total cost and (2) the medication cost for both preventive and curative use were evaluated. Medication costs 1, 3, 6, 12, 24, 36 and 48 months after RYGB were compared with those at baseline. Two-way ANOVA and chi-square tests were used. Statistical significance was set at $p<0.05$.

9.3 Results

We included 143 patients whose characteristics are displayed in Table 29. BMI gradually decreased during the first year post-RYGB and then stabilized up to 4 years (48.6±9 vs. 33.2±8 kg/m²; Figure 16) (i.e., mean weight loss, 33±18 kg).

Table 29: Characteristics of the study cohort at baseline before RYGB surgery

Patients characteristics	
Total	143
Female, *n* (%)	112 (78.3%)
Age (year), mean±SD	42.9±12
BMI (kg/m²), mean±SD	48.6±8.8
Obesity related diseases *n* (%)	
Type 2 diabetes	49 (34%)
Obstructive sleep apnea	47 (33%)
Cardiovascular disease	71 (50%)
Dyslipidemia	38 (27%)
Psycho- and neurological disorders	44 (31%)

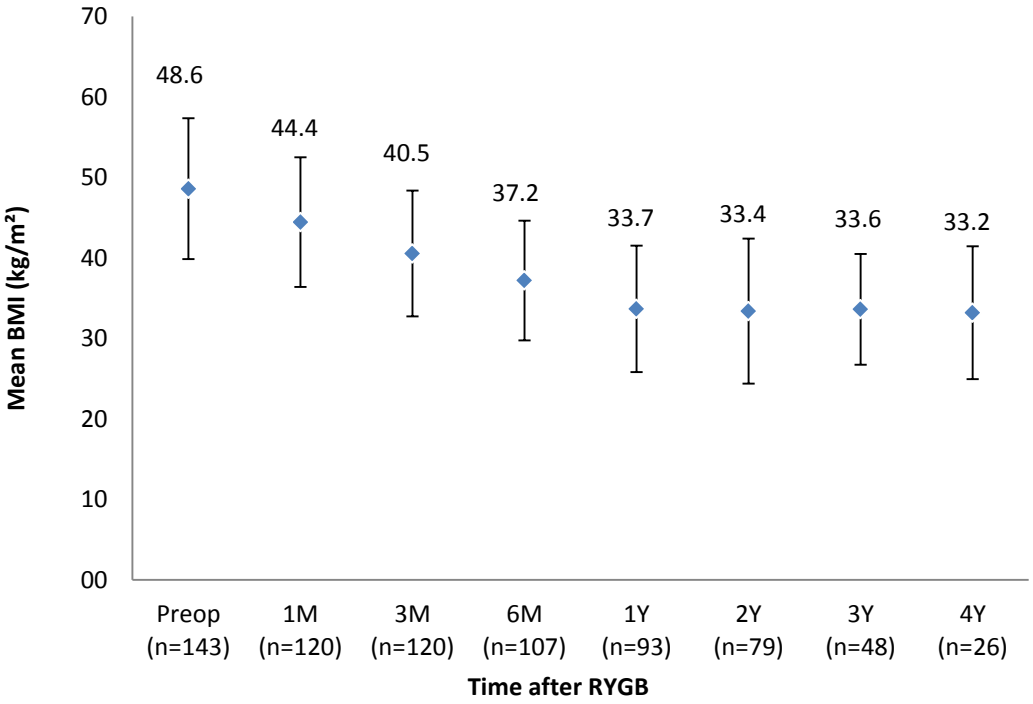


Figure 16: Mean BMI±SD before and after RYGB. On the *x*-axis, the number of patients at each time point of the follow-up is represented. *(preop* before surgery, *1M* 1 month*, 1Y* 1 year*)*

When compared to baseline values, we observed a significant 32% reduction in the total prescription costs 1 year post-surgery (p<0.01; Figure 17). Curative medications and medical device cost significantly decreased as early as 1 month post-surgery and onwards (Figure 18). The most significant reduction in the mean prescription cost concerned T2DM and OSA treatments (Table 31). Indeed, 34% of patients were treated for T2DM at baseline, which was reduced respectively to 16, 11, 9 and 0%, after 3 months, 1, 2, and 4 years after RYGB. Likewise, T2DM treatment cost decreased by 65% (p<0.01) at 1 month, and further over time to reach 0€ 4 years post-RYGB (Table 30).

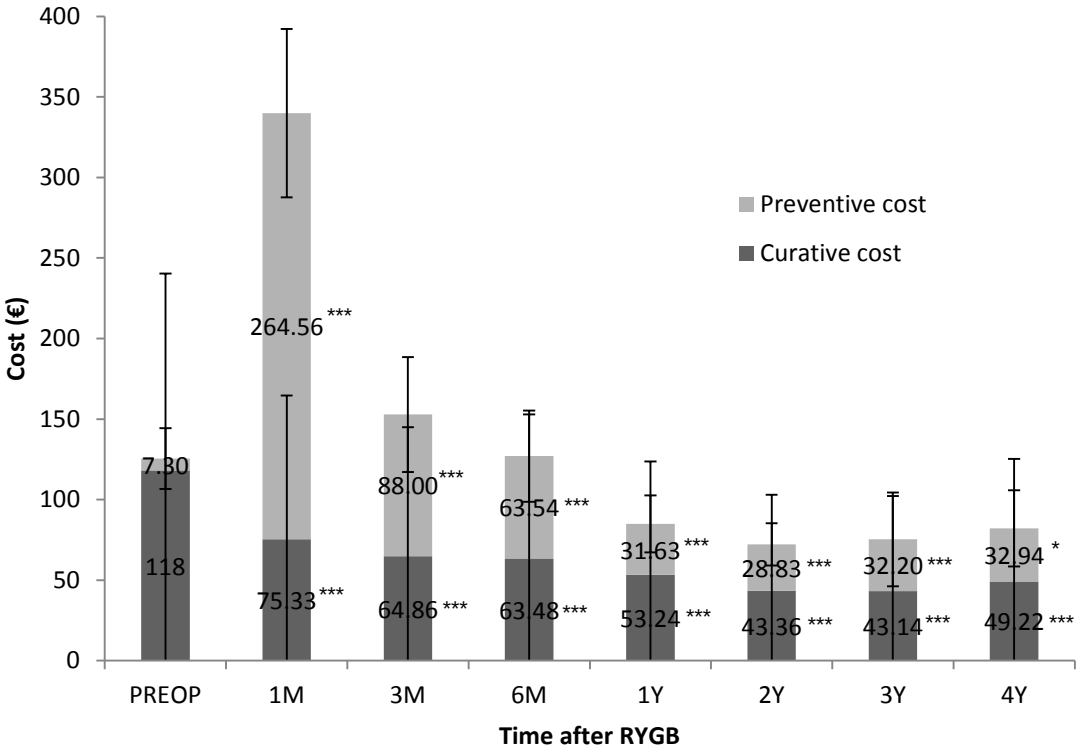


Figure 17: Medication cost per month per person pre-and post-RYGB, subdivided in preventive and curative medication use; significant differences are shown in comparison with baseline costs
(preop before surgery, *1M* 1 month*, 1Y* 1 year*)* * $p<0.05$; ***; $p<0.001$

Table 30: The different treatments for T2DM before and after RYGB along the follow-up

Therapy	Baseline	1M	3M	6M	1Y	2Y	3Y	4Y
Monotherapy (*n*) (oral antidiabetic (OAD)	15 (31%)	7 (33%)	7 (37%)	5 (38.5%)	3 (30%)	3 (43%)	2 (67%)	0
Bitherapy (oral antidiabetic)	10 (20%)	0	2 (10%)	2 (15.5%)	3 (30%)	2 (28.5%)	1 (33%)	0
Tritherapy (oral antidiabetic)	1 (2%)	0	0	0	0	0	0	0
OAD + insulin	20 (41%)	4 (19%)	4 (21%)	3 (23%)	3 (30%)	2 (28.5%)	0	0
Insulin only	3 (6%)	10 (48%)	6 (32%)	3 (23%)	1 (10%)	0	0	0

Table 31: Medication cost per month (at each time point post RYGB compared to baseline)

Comorbidity	Number of patients at baseline	Average medication cost per month at baseline (€)	1 month post surgery (€)	3 months post surgery (€)	6 months post surgery (€)	1 year post surgery (€)	2 years post surgery (€)	3 years post surgery (€)	4 years post surgery (€)
Type 2 Diabetes	Patients with the comorbidity (*n*=49)	81.0	-52.6*** [-76.6;-28.7]	-63.3*** [-87.3;-39.4]	-66.0*** [-91.2;-40.8]	-69.2*** [-94.7;-43.7]	-67.4*** [-95.1;-39.6]	-75.35***[-107.6;-43.1]	-81.0*** [-131.0;-31.1]
	All patients (*n*=143)	28.3	-20.2*** [-37.2;-3.2]	-23.3*** [-40.1;-6.6]	-24.2*** [-41.6;-6.9]	-24.9*** [-43.0;-6.8]	-24.48***[-43.55;-5.4]	-26.7*** [-49.3;-4.1]	-28.3** [-57.2;0.55]
Obstructive sleep apnea	Patients with the comorbidity (*n*=47)	115.7	-14.1 [-36.9;8.7]	-21.5** [-44.3;1.3]	-50.8*** [-73.9;-27.7]	-72.7*** [-96.6;-48.8]	-91.8***[-117.2;-66.3]	-102.8***[-132.6;-73.1]	-101.2***[-142.1;-60.4]
	All patients (*n*=143)	35.6	0.6 [-16.4;17.6]	-1.8 [-18.6;14.9]	-10.7 [-28.0;6.6]	-19.2*** [-39.4;-1.1]	-26.7*** [-45.8;-7.6]	-30.8*** [-53.4;-8.2]	-31.2*** [-60.0;-2.3]
Cardiovascular diseases	Patients with the comorbidity (*n*=71)	33.2	-3.9 [-23.0;15.2]	-9.4 [-28.5-9.7]	-7.4 [-27.0;12.1]	-13.6 [-33.6;6.4]	-17.1* [-37.7;3.4]	-12.3 [-36.4;11.8]	-5.5 [-37.6;26.6]
	All patients (*n*=143)	16.5	-2.7 [-19.7;14.2]	-5.9 [-22.3;11.3]	-4.0 [-21.3;13.4]	-6.2 [-24.3;11.9]	-7.4 [-26.5;11.7]	-4.3 [-26.9;18.3]	-1.6 [-30.5;27.3]
Dyslipidemia	Patients with the comorbidity (*n*=38)	27.1	-5.6 [-31.3;20.2]	-14.7 [-40.5;11.0]	-17.9 [-43.6;7.9]	-22.0* [-49.1;5.1]	-20.6 [-49.5;8.3]	-16.8 [-49.2;15.6]	-19.4 [-66.1;27.4]
	All patients (*n*=143)	7.2	-1.8 [-18.7;15.2]	-4.0 [-20.8;12.8]	-4.5 [-21.8;12.8]	-5.81 [-23.9;12.3]	-5.4 [-24.5;13.6]	-4.0 [-26.6;18.6]	-5.2 [-34.1;23.7]
Psycho- and neurological disorders	Patients with the comorbidity (*n*=44)	18.0	-5.0 [-28.5;18.6]	-5.3 [-28.8;18.3]	-2.3 [-26.8;22.2]	0.7 [-25.0;26.5]	-2.0 [-29.4;25.3]	-5.9 [-36.9;25.2]	7.7 [-29.6;44.9]
	All patients (*n*=143)	5.5	-1.5 [-18.4;15.5]	-1.2 [-18.0;15.6]	0.5 [-16.8;17.8]	1.6 [-16.5;19.7]	0.4 [-18.7;19.5]	-1.0 [-23.6;21.6]	5.3 [-23.6;34.2]
Total curative cost	All patients (*n*=143)	118.2	-42.8*** [-59.8;-25.9]	-53.3*** [-70.1;-36.5]	-54.7*** [-72.0;-37.4]	-64.9*** [-83.0;-46.8]	-74.8*** [-93.9;-55.7]	-75.0*** [-97.6;-52.4]	-68.9*** [-97.8;-40.0]

The mean difference between the medication cost per month (at each time point post RYGB compared to baseline) and the preoperative cost per month with the 95% confidence interval. The total number of patients was 143.

*p<0.05; ** p<0.01; *** p<0.001

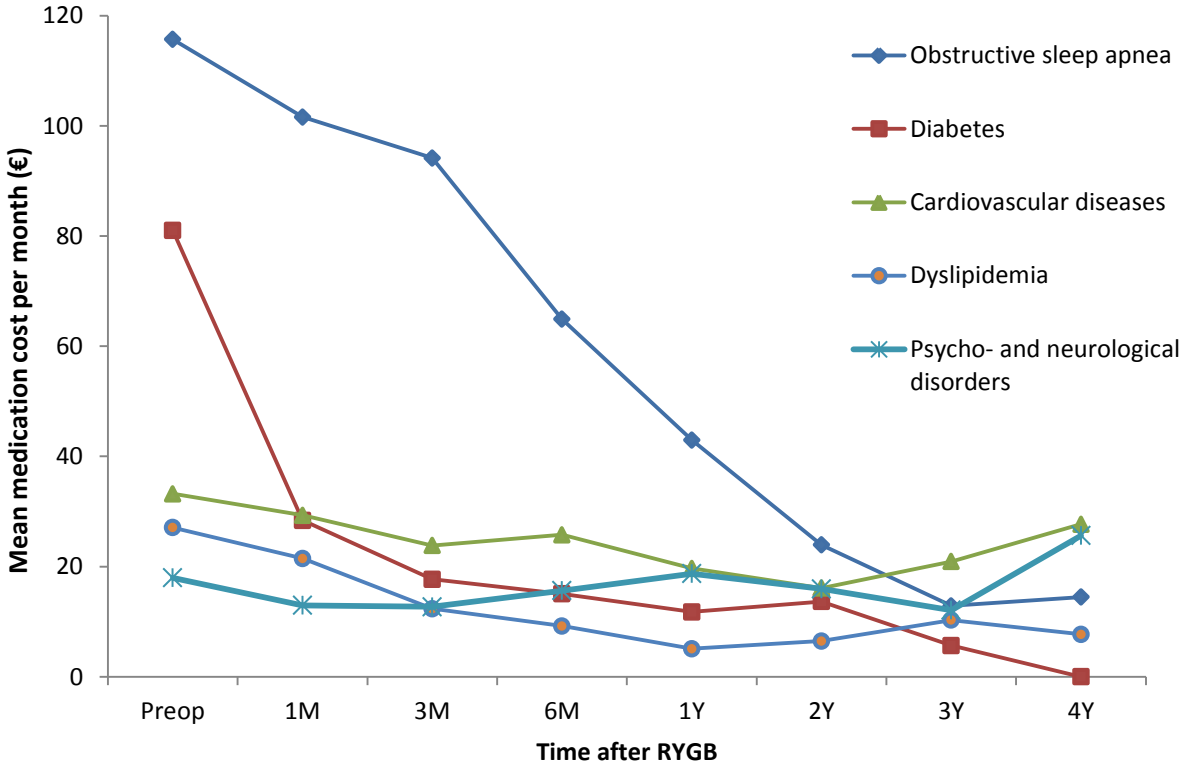


Figure 18: Mean cost per patient per month for the different comorbidities, among the patients who suffer from the comorbidity *(preop* before surgery, *1M* 1 month*, 1Y* 1 year*)*

OSA mean treatment cost decreased significantly as early as 3 months post-surgery and onwards to reach its lowest cost (12.86 €/month) at 3 years (i.e., 89% reduction vs. baseline). By contrast, drugs for CVD and dyslipidemia's cost decreased more weakly (-51.5 and -81%, respectively), only reaching significance 1 and 2 years post-RYGB. We observed no significant change regarding other obesity-related disease prescriptions.

As expected, preventive treatment's prescription just before the surgery induced a 36-fold increase in the cost 1 month post-surgery, compared with baseline. Although this cost decreased over time, but it remained significantly higher than baseline values, even after 4 years.

9.4 Discussion

This study aimed to investigate the overall medication costs after RYGB. We focused both on the costs of the different obesity-related diseases treatments, and those of treatments prescribed to prevent surgery-related complications, and observed a significant reduction in the global medication

cost 1 year post-surgery and onwards, compared with baseline. This major post-surgery cost reduction was mainly explained by the acute decrease in T2D and OSA treatment prescriptions.

The marked reduction in T2DM medication use observed in our study, resulted in an 85-fold decrease in medication cost 1 year post-RYGB among the patients with diabetes, which is in line with previous pharmaco-economic studies. Segal et al. [53] evaluated medication use in 6,235 patients undergoing bariatric surgery and demonstrated 76% reduction in T2DM medication use 1 year post-surgery that persisted up to 4 years [209]. A significant reduction in oral antidiabetic agents and insulin therapy (80 and 79%) 4 years post-RYGB was also observed in another study, including 191 diabetic patients [210]. Although our cohort was of a smaller size, the reduction of T2DM treatment is in line with a significant improvement or normalization of both HbA1c and fasting glycemia during follow-up. Literature entails that RYGB enables (1) a 57.5% T2DM remission [211] according to the definition [212], (2) a 84% improvement in T2DM status [213], and (3) a reduction in the occurrence of T2DM [214]. We herein demonstrate a significant cost reduction as soon as 1 month post-RYGB, in agreement with the rapid improvement of diabetes post-surgery which involves multiple mechanisms [215-217], some unrelated to weight loss [210;215;218].

OSA occurred in 33% of our patients at baseline. We observed a 63% reduction in the cost of CPAP 1 year post-RYGB, in line with previous studies [4;219-222]. Likewise, we confirmed the possibility to stop CPAP upon clinical examination and polysomnography. In contrast with T2DM, OSA improvement only becomes apparent when substantial reduction in BMI occurs, suggesting the importance of maintained weight loss. Yet, as acknowledged by studies comparing the effects of surgical versus diet-induced weight loss [223], the improvement of OSA involves numerous mechanisms other than weight loss [224].

Bariatric surgery reduces all-cause mortality, particularly death from CVD [202;225;226] and improves CVD risk factors [10]. We herein confirm the decrease in medication cost for dyslipidemia and CVD, reaching significance 1 and 2 years post-RYGB. However, these reductions were not significantly

sustained on the longer term, although our patients did not regain weight over the 4 years follow-up (Figure 16). Previous reports showed that although 51 and 59% of the patients could stop their hypertension or dyslipidemia medications soon post-surgery [227;228], the incidence of both diseases tends to increase again on the longer term (2 and 10 years) [202]. The fact that in our study drugs that were used for secondary prevention of cardiovascular events were pooled with drugs for hypertension and CVD could be another reason for the observation that reductions in drug costs for CVD did not sustain over time. Secondary prevention treatment is expected not to be discontinued, even if clinical or biological parameters improve.

By contrast, we did not observe a significant decrease in psychoneurological treatment costs, in line with previous reports [229]. Indeed, no significant change in the prevalence of antidepressant prescription was observed in a retrospective study including 439 patients before and post-RYGB [229]. Likewise, we recently demonstrated that the physical, but not the mental component of quality of life significantly improved 1 year after surgery [230].

While medication costs for curative use decreased, preventive medication costs increased immediately post-surgery. This can be explained by the choice of prevention regimen in our department [206]. Although the cost for preventive medication decreased over time, daily minerals and vitamins should never be discontinued as the prevention of nutrient deficiencies is lifelong [206].
Overall, the strength of our study lies in the fact that the evolution of treatment costs for obesity-related diseases was confronted with clinical examination and biological parameters obtained at each follow-up time point. This highlights the need for frequent medical follow-up enabling health care professionals to adapt the treatment to patients' needs in order to avoid side effects, such as hypoglycemia with antidiabetic drugs. Furthermore, these patients should receive sufficient information about their medication use, and need frequent adaptation of the medication scheme after surgery to provide correct adherence. We deliberately chose to limit our analysis on the impact

of RYGB only, since different bariatric surgery techniques induce different results in terms of obesity-related diseases improvement or remission, thus impacting on drug associated costs.

However, our study presents some limitations. The medication classification in preventive and curative use, and the different comorbidities, is rather arbitrary. Besides, the recruitment of patients in a university hospital reflects the most severe obese patients with worse conditions which might reinforce the beneficial cost's reduction we herein observe. To generalize our findings, our results need to be verified in a more general population of candidates to RYBG to validate whether they still hold true.

Finally, we decided to focus on treatment costs and did not address other costs related to surgery follow-up, such as hospital days and non-primary care visits, thus we cannot conclude on the total health care costs. Some recent studies have however addressed this issue. The SOS study demonstrated that hospital days and visits were significantly reduced from 7 years and onwards after surgery when comparing surgery to non-surgery obese patients [54]. By contrast, Weiner et al. failed to observe any difference in overall health care costs between surgery patients and a matched obese group [231]. Of note, in those two studies, multiple bariatric surgeries were assessed including techniques that are known to induce surgical reoperations such as lapband and vertical gastroplasty.

9.5 Conclusions

In this study, we took into account medications prescribed for obesity-related diseases and for prevention of surgical and nutritional complications, thus reflecting everyday life post-RYGB. Our study shows that RYBG induces a significant reduction of total medication costs, as early as 1 year post RYGB, compared to baseline. Most importantly, the reduced medication cost was maintained over time several years post-RYGB and appears mainly to be due to the significant improvement of T2DM and OSA. Hence, RYGB entails economic benefits by reducing the costs and need for medication and medical devices.

PART VI: CURRENT CLINICAL PRACTICE

CHAPTER 10: BARRIERS IN THE APPROACH OF OBESE PATIENTS UNDERGOING BARIATRIC SURGERY IN FLEMISH HOSPITALS

This chapter is based on:
Gesquiere, I., Augustijns, P., Lannoo, M., Matthys, C., Van der Schueren, B., Foulon, V. (2015)
Barriers in the approach of obese patients undergoing bariatric surgery in Flemish hospitals
Obesity Surgery; DOI: 10.1007/s11695-015-1680-0

(with permission from Obesity Surgery)

10 BARRIERS IN THE APPROACH OF OBESE PATIENTS UNDERGOING BARIATRIC SURGERY IN FLEMISH HOSPITALS

Background*:* Bariatric surgery is associated with weight loss and improvement of comorbidities of obesity but also with short and long-term complications. Preoperative screening and lifelong follow-up of these patients is important to optimize the effect of bariatric surgery and minimize complications. The objective of this study was to create an inventory of the current care offered to bariatric patients before and after surgery in Flemish hospitals, Belgium and to identify barriers for optimal care.

Methods: Semi-structured interviews with health care professionals involved in screening and follow-up of bariatric patients in 12 hospitals in Flanders, Belgium were performed. Interviews were transcribed verbatim and analyzed with NVivo 10.0.

Results: In each participating hospital, except one, biochemical screening before and after bariatric surgery was performed, but the extent and timing varied between the hospitals. In 10 hospitals, a standard multivitamin preparation was started in all patients after surgery, but there was a large variation for timing of initiation and duration between hospitals. The interviewees indicated that the knowledge about appropriate dosage and formulation adjustments after surgery was limited. Most of the performed drug adjustments were due to improvement of comorbidities. In 9 out of 12 hospitals, a multidisciplinary team was involved, but the approach varied widely. Only in 3 out of 12 hospitals, eligibility of patients for bariatric surgery was discussed in team meetings.

Conclusions: Strategies to implement existing guidelines are required in order to obtain more uniform, interdisciplinary support for bariatric patients, resulting in an increase of efficiency of surgery and improved patient care.

10.1 Introduction

Over the last decades, the prevalence of obesity has increased to epidemic proportions [1]. This is associated with an increase in the number of performed bariatric surgeries, currently the only way to achieve major and sustainable weight reduction in morbid obese patients [141]. Bariatric surgery has also beneficial effects on certain comorbidities of obesity such as type 2 diabetes, hypertension, and sleep apnea [4]. However, both short and long-term complications can occur; and nutritional deficiencies often arise [11]. Furthermore, the absorption of oral administrated drugs can be altered after bariatric surgery, which may cause under- or overdosing resulting in serious therapeutic consequences [58;232]. To minimize complications, screening and follow-up by a multidisciplinary team that has knowledge about changes and problems after bariatric surgery are essential [233-235].

In Belgium, patients older than 18 years with a BMI≥40 kg/m² (or BMI≥35 kg/m² associated with comorbidities) receive reimbursement of the costs for bariatric surgery from the National Institute for Health and Disability Insurance (NIHDI) if they followed a documented diet during one year and obtained approval from the surgeon, internist, and psychologist/psychiatrist. However, no follow-up consultations are required for the reimbursement of the surgery. Moreover, there is no reimbursement of screening and follow-up consultations.

The American Association of Clinical Endocrinologists/the Obesity Society/American Society for Metabolic and Bariatric Surgery (AACE/TOS/ASMBS) and the European Chapter of the International Federation of Surgery (IFSO-EC)/European Association for the Study of Obesity (EASO)/EASO Obesity Management Task Force (EASO OMTF) have designed guidelines concerning bariatric surgery, including screening and follow-up [36;236], but to what extent these guidelines are followed in clinical practice is not known.

The objective of this study was therefore to create an inventory of the current care offered to bariatric patients before and after surgery in Flemish hospitals, Belgium and to identify barriers for optimal care.

10.2 Methods

10.2.1 Setting and sampling

Twelve hospitals that were selected by purposive and convenience sampling participated in this study. All were based in Flanders, Belgium. We included 3 small, 5 medium and 4 large bariatric centers (< 100, 100-200 and > 200 bariatric surgeries/year, respectively). In all participating hospitals, semi-structured interviews were performed with health care professionals (HCPs) (e.g. surgeons, endocrinologists, psychologists, dietitians, cardiologists) involved in the screening and follow-up of bariatric patients during the period July 2013 – February 2014. Informed consent was obtained from all individual participants included in the study. Ethical approval was obtained from the Medical Ethics Committee of the University Hospitals Leuven (ML8339) and all procedures were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards

10.2.2 Data collection

Four researchers conducted the semi-structured interviews. Based on information from literature and direct observation in a hospital, an interview guide, consisting of 3 major topics, was compiled: prevention and follow-up of nutritional deficiencies, medication, and multidisciplinary counseling. All interviews were performed in Dutch, the mother tongue of the interviewees. The interviews were audio recorded and transcribed verbatim, after which the recordings were removed.

10.2.3 Data analysis

Four investigators discussed the transcribed interviews to develop a coding scheme in NVivo 10.0. The transcripts were then reread and coded by three researchers. A coding comparison test was performed to determine inter-rater variability and degree of agreement between coders. The overall mean kappa coefficient was 0.96 and the percentage of agreement was 98.39%. Quotes mentioned in the text have been translated from Dutch to English by a certified translator.

10.3 Results

In this study, 45 HCPs involved in the screening and follow-up of bariatric patients were interviewed in 12 different hospitals. The characteristics of the hospitals and interviewees and the number of hospitals following the main recommendations from existing guidelines, are summarized in Table 32 and Table 33.

10.3.1 Nutritional evaluation

Regarding follow-up of nutritional deficiencies, each hospital, except one, performed pre- and postoperative biochemical screening. However, the extent and timing of the screening varied between hospitals and 25% of the hospitals screened only once after surgery. In one hospital, diagnosis of postsurgical nutritional deficiencies was only based on clinical symptoms.

Remarkably, in 9 hospitals, blood collection for follow-up was performed by the general practitioner (GP), while the results were discussed with the surgeon. In one hospital, the GP was responsible for the entire follow-up of deficiencies. The interviewees indicated drop out of patients for follow-up occurred frequently, despite the fact that the hospitals provided follow-up consultations.

In ten hospitals, a multivitamin preparation was systematically started in all patients after surgery. In one of the other hospitals, multivitamins were only started if patients developed a deficiency; in the other hospital, all patients received a supplement of vitamin D and calcium instead of a multivitamin preparation. We further observed that the timing of initiating a multivitamin preparation varied between a few days to six months after surgery and that the duration varied from one year to lifelong (in 1/3 of the hospitals). The opinions on the value of lifelong vitamin supplementation varied; some interviewees supported it *"I think it is of vital importance to take extra vitamins. When I draw up what these people eat, they never reach their required amount of vitamins,*" while others found it useless *"That's some very expensive urine. Because half of what you ingest, you simply pee out again.*" All hospitals initiated specific micronutrient supplements upon the development of a nutrient deficiency (both pre- and postoperatively).

Table 32: Characteristics of the hospitals (H) and interviewees

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
Hospital												
Size (small S, medium M, large L)	M	S	M	M	S	S	L	L	L	M	L	M
MD team	x	x	x	x	x	x	x	x	x	0	x	x
Interviewee												
Surgeon	x	x	x	x	x	x	x	x	●	x	x	x
Psychologist	x	x	x	x	x		x	x	x		x	
Dietitian	x	x		x	x	x	x	x	x		x	x
Endocrinologist				x	x	x	x	x	x			x
Psychiatrist			x			x				x		x
Cardiologist	x											
Gastroenterologist					x							
Nurse							x			x		
Clinical pharmacist						x						
Physiotherapist								x				

X interviewed
0 not present in hospital
● involved in screening and follow-up of bariatric patients, but not interviewed

Table 33: Number of hospitals following recommendations from existing guidelines

Recommendation	Number of hospitals
Biochemical screening of nutritional deficiencies preoperative [36;236]	12/12
Biochemical screening of nutritional deficiencies postoperative [36;236]	11/12
Standard initiation of a multivitamin preparation postoperative [36;100]	10/12
Early postoperative care: guidance by a registered dietitian [36]	7/12
Presence of multidisciplinary team [36;236]	9/12

10.3.2 Medication

All hospitals inquired actual medication use before surgery. In one hospital, a clinical pharmacist was responsible for this part of the assessment; in the other hospitals, it was done by the physician/surgeon. Many interviewees indicated that their knowledge about appropriate dosage and formulation adjustments after bariatric surgery is very limited. They acknowledged that adjustments of drug dosages were mostly due to the improvement of comorbidities. Dosage adjustments due to altered pharmacokinetics were not performed, unless patients showed unexpected side effects or less effect of the drug. In a minority of the hospitals, medication with an extended release or a large size was systematically replaced by an alternative.

After surgery, in at least 9/12 hospitals, a proton pump inhibitor (PPI) was systematically initiated. Furthermore, most HCPs reported to avoid medication that increases the risk of gastrointestinal ulceration such as NSAIDs and corticosteroids.

Most surgeons referred to the GP and allied specialized physician for follow-up of patients' medication use as they do not want to undermine the role of HCPs in primary care. "*We don't want to fight over it. We shouldn't create the feeling that bariatric surgery is a direct ticket to the hospital.*"

10.3.3 Multidisciplinary approach

In 9/12 hospitals, the multidisciplinary team involved in the care of bariatric patients consisted of at least four members: surgeon, internist, dietitian, and psychologist/psychiatrist. However, there was a large variation in multidisciplinary approach between the hospitals. In 3/12 hospitals, eligibility for surgery was discussed in meetings with all team members. In other hospitals, eligibility of doubtful cases was either discussed in direct contact or contact by telephone; in other cases, the medical

record of the patient served as an exchange platform. In two hospitals, the medical record was the only means of exchange of information.

In the hospitals where multidisciplinary meetings were established, "*For me it's the combination, the multidisciplinary nature, the combination of being open to each other, exchanging ideas. That for me is the complete approach and the patient should notice this."* These interviewees also claimed that without effective communication between HCPs, it is difficult to know what has already been discussed with the patient and whether patients are telling the truth: "*Because beware: patients can be very naughty at such instances. Some have the guts to simply go to the surgeon and lie. To state: 'Yes, but I've been there and everything was ok'."* Others found no added value for a multidisciplinary approach: "*Actually I don't have much time for that, but then I again, when I see that everyone agrees, I also think it is useless.*" Interviewees mentioned lack of time and budget and distance between different departments of the hospital as possible causes of the low implementation of multidisciplinary meetings.

Remarkably, in 3/12 hospitals, the dietitian was not involved in the preoperative screening. In these hospitals, the role of the dietitian was thought to be replaced by another team member, but this was not done consistently. Furthermore, only in seven hospitals, there was a standard follow-up consultation by the dietitian and only in two hospitals by a psychologist. One hospital had a clinical pharmacist in the multidisciplinary team (only involved preoperatively).

10.4 Discussion

The interviews with HCPs involved in screening and follow-up of bariatric patients shows that the approach of obese patients before and after bariatric surgery varies widely between hospitals.

10.4.1 Nutritional evaluation

The clinical practice guideline composed by AACE/TOS/ASMBS states that there is strong evidence for appropriate nutritional evaluation of all patients who will undergo bariatric surgery [36]. This was also

highlighted in recent studies, emphasizing on screening and lifelong follow-up for nutritional deficiencies [36;233]. The current study shows that preoperative biochemical screening was performed in all participating hospitals, although often very limited. Besides, there was a wide variation in timing of follow-up consultations, which were often performed in cooperation with the GP. Hence, effective communication between primary and secondary care is necessary. Furthermore, GPs need sufficient knowledge regarding the impact of bariatric surgery.

The guidelines from ASMBS and from the Clinical Guidelines Subcommittee of the Endocrine Society both recommend the long term use of multivitamins after bariatric surgery. Additional supplementation is required in many patients; the extent of which depends on the type of surgery. However, up till now, the exact need of additional supplementation is not known [36;100;125]. In the current study, we found a wide variation between hospitals for timing of initiation and duration of multivitamin supplementation. As interviewees indicated that they have insufficient knowledge about supplementation of micronutrients, more training and standardization is required.

10.4.2 Medication

Patients who will undergo bariatric surgery should receive information about the influence of the surgery on medication use to avoid medication related problems [237]. Bariatric surgery alters the anatomical structure of the gastrointestinal tract, which has an impact on drug bioavailability. The clinical effect of the potentially altered drug bioavailability is for most drugs currently unknown. This limited knowledge about medication adjustments after bariatric surgery was also mentioned by many interviewees.

The improvement of comorbidities after bariatric surgery and the concomitant improvement in medication use for these comorbidities are well known [52;238]. Regarding drug use, the guidelines of ASMBS therefore recommend a repeated evaluation of the comorbidities to adjust the associated drug use, as well as the avoidance of the use of NSAIDs [36]. Both guidelines were followed in most of the participating hospitals.

In the current study, only a minority of HCPs took into account the formulation of drugs and supplements upon prescribing. From a theoretical point of view, it is suggested to avoid medication with an extended release [57]; however, up till now, no comparative studies have been performed with a controlled release formulation after RYGB to underpin this recommendation. Furthermore, it is recommended to change some formulations early after bariatric surgery, e.g., tablets should be crushed or changed to a liquid formulation for 3-8 weeks after surgery [239]. In the ASMBS guidelines, a chewable form of micronutrient supplements is therefore recommended during the first 3-6 months after surgery [36]. In our study, only in two hospitals supplements were initiated in a liquid formulation.

10.4.3 Multidisciplinary approach

Different papers have already highlighted the need for a multidisciplinary approach of patients with bariatric surgery in order to provide more uniformity and to improve preoperative screening and postoperative care [240-242]. The main advantage of a multidisciplinary team is providing a continuum of care to optimize outcomes and minimize complications [243].

The current study showed that there was a large variation in multidisciplinary approach (e.g. members of the team, communication) among the participating hospitals. This is in line with a previous survey performed by Santry et al. in the USA [241]. In the hospitals with established multidisciplinary meetings, the interviewees experienced better communication. Communication is essential for safe care in patients undergoing bariatric surgery [243].

The Interdisciplinary European Guidelines advise to involve a dietitian and/or nutritionist in the multidisciplinary team [236]. Registered dietitians can help patients making informed choices regarding bariatric surgery, and they can educate and counsel them during postoperative follow-up as adjustment of their eating behavior and lifestyle is required to obtain optimal outcomes of surgery [244-246]. The ASMBS recommends that all patients who will undergo bariatric surgery should have a proper nutritional evaluation [10]. Although dietitians are specialized in nutritional assessment and

counseling, not all participating hospitals engaged them in screening and follow-up. Moreover, interviewees indicated that patients often not maintained counseling by a dietitian and suggested that reimbursement of these consultations could facilitate long-term follow-up.

Likewise, a psychosocial evaluation before and after bariatric surgery has been shown to optimize the outcomes of bariatric surgery [247;248]. In all participating hospitals, a psychologist/psychiatrist was involved in the multidisciplinary team. This result, which is in contrast with the data of a study performed by van Hout et al. in The Netherlands, is probably due to the fact that screening by a psychologist/psychiatrist is required for reimbursement of the surgery in Belgium [249].

Only in one hospital, a clinical pharmacist was involved in the multidisciplinary team. Nevertheless, Silverman et al. have shown that collaboration between surgeons and pharmacists improved pharmaceutical care in these patients as the pharmacist suggested to crush medication and to change the formulation and gave other relevant pharmaceutical advice [250].

The barriers to implement a multidisciplinary approach mentioned in literature and raised during the interviews are comparable, with lack of time and budget as the most important [22]. To promote a true multidisciplinary approach, a compensation for multidisciplinary meetings should be provided. Furthermore, reimbursement of follow-up consultations could help. A recent review performed by Kim et al., has shown that gastric bypass surgery resulted in a greater weight loss when patients attended follow-up appointments [251].

10.4.4 Recommendations

Based on these findings, we believe there is a need for strategies to implement existing guidelines in order to offer multidisciplinary support for bariatric patients. Moreover, HCPs indicate they have insufficient knowledge of how to adapt drug regimens or nutritional support following surgery. Thus, more studies are needed to increase knowledge about micronutrient supplements and the influence on medication absorption.

10.4.5 Strengths and limitations

The included hospitals were spread over Flanders and consisted of large and small centers, assuming representativeness for Flanders. Current practice in other countries, however, may differ from these observations and may be closer to or further from implementation of the guidelines. The advantage of the performed qualitative interviews is that it gives insight in participants' feelings and expectations, hence, allowing an exploration of strengths and weaknesses in current care.

10.5 Conclusions

Current care for bariatric patients in Flemish hospitals, both before and after surgery, varies widely. Strategies to implement existing guidelines are required in order to obtain more uniform, multidisciplinary support for bariatric patients, resulting in an increase of efficiency and improved patient care.

PART VI: GENERAL DISCUSSION

11 DISCUSSION AND FUTURE PROSPECTIVES

The overall aim of this PhD project was to investigate the effect of Roux-en-Y gastric bypass on dietary intake, medication and supplement use and oral drug bioavailability. In this chapter, the main findings of the project will be discussed, recommendations for clinical practice will be provided and the methodological considerations of this PhD project will be argued, including strengths and limitations. In the last part, future perspectives will be presented.

11.1 General discussion of findings

The population group with a bariatric history is growing enormously. In Belgium, yearly more than 10,000 people undergo bariatric surgery to lose weight; this number has been doubled over the last five years. However, there is still a lot of information missing to be able to provide patients with an optimal, patient-specific follow-up; for example, evidence based guidelines regarding pharmacotherapy are un-existing. This observation was the starting point of the current PhD project, in which we performed studies with patients before and after surgery, and with health care professionals (HCPs) involved in the screening and follow-up of these patients.

FOLLOW-UP OF BARIATRIC PATIENTS VARIES WIDELY

The qualitative interviews that were performed with HCPs in Flemish hospitals have shown that the follow-up of patients after RYGB varies widely. The interviewed HCPs confirmed that the current knowledge regarding an adequate clinical follow-up, including nutritional recommendations and drug dose adjustments, is insufficient. Hence, these interviews underpinned the necessity of the current PhD project that aimed to broaden the knowledge regarding these topics in order to implement the added knowledge in clinical practice.

RYGB HAS A MAJOR EFFECT ON INTAKE OF MACRO- AND MICRONUTRIENTS

From the prospective study, we learned that the energy and macronutrient intake was the highest before surgery and decreased enormously one month post-RYGB; thereafter the intake gradually

increased again, even up to the baseline intake of some individuals. This pattern is consistent with previous studies [17;28;40-42]. The dietary intake of iron, vitamin B_{12}, vitamin C and copper also decreased enormously one month post-RYGB, and gradually increased until 12 months post-RYGB; however, intake of all micronutrients remained below baseline values [40;46;48]. The dietary intake of zinc was lowest 3 months post-RYGB, and then started to increase again.

Because of the reduced food intake, the prevalence of inadequate dietary intake of macro- and micronutrients, compared to the age- and gender- specific Estimated Average Requirements (EAR) or Adequate Intake (AI) [105] increased after surgery, especially the first months after RYGB. One year post-RYGB, this percentage was still very high for fiber and vitamin C. Even when adding the supplement intake to the dietary intake of the different micronutrients, there were still some patients with an inadequate intake at the different time points, except for vitamin B_{12} at 3 and 12 months postoperatively and for zinc 12 months postoperatively.

RYGB RESULTS IN WEIGHT LOSS AND CHANGES IN BODY COMPOSITION

The goal of bariatric surgery is losing weight; obviously we also observed changes regarding body composition in the patients participating in the prospective study. A significant decrease in FMI until one year and FFMI until 6 months post-RYGB was observed. These results are consistent with previous studies [118-120]. Furthermore, a significant correlation between protein intake and FFMI was identified, confirming the importance of protein intake during weight loss to preserve lean mass. However, we need to take into account that no data regarding physical activity were collected and that physical activity also has an important influence on lean body mass.

RYGB IMPROVES COMORBIDITIES AND REDUCES DRUG USE

Bariatric surgery not only results in weight loss, but also in an improvement of a lot of comorbidities, hence influencing drug use [9;52-54]. In a retrospective study, we have confirmed that the overall medication costs (i.e. both the cost associated with medication and medical devices for preventive and for curative use) decreased one year after RYGB and onwards. Moreover, we included the cost

associated with medication and medical devices for preventive and curative use and we observed a significant reduction in the global medication cost 1 year post-surgery and onwards, compared with baseline. This major post-surgery cost reduction was mainly explained by the acute decrease in T2D and OSA treatment prescriptions. This was in contrast with the preventive medication costs, which increased immediately post-surgery.

IRON STATUS IMPROVES AFTER RYGB

The iron status in the patients included in the prospective study improved after RYGB through an increased transferrin saturation and a decreased concentration of C-reactive protein (CRP), ferritin and hepcidin; this decrease is associated with the improvement of the obesity-associated low-grade systemic inflammation [131]. The latter is a potential indication for enhanced iron absorption after RYGB and subsequently for an improved iron profile.

However, the results of the prospective study with regard to the iron status (Chapter 4) are not completely consistent with the results of the retrospective study on iron deficiency in RYGB patients (Chapter 5). This could be explained by the fact that these studies were performed during two different time periods as shown in Figure 19. In the time period between both studies, a lot of changes have been introduced in daily clinical practice at the University Hospitals Leuven with respect to the management of RYGB patients. The follow-up by different HCPs has been optimized and there is an increasing trend to advice patients with a bariatric surgery history to take vitamin/mineral supplements. The latter probably contributes to the improved iron status post-RYGB, observed in the prospective study as an increase in transferrin saturation and a decrease in hepcidin concentration one year post-RYGB. The retrospective study was carried out on data of patient visits that took place between 2006 and 2010. In this period, supplement use was less frequent, which is reflected by the less favorable iron status in these patients.

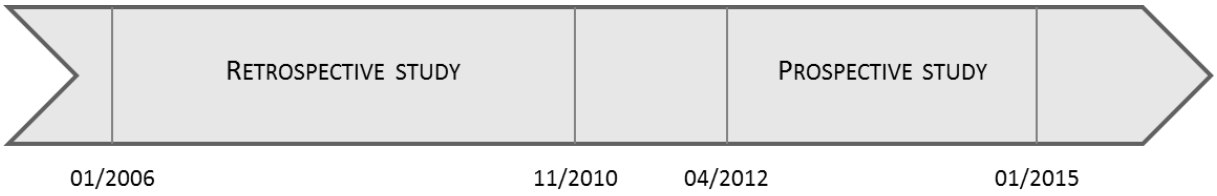


Figure 19: Time frame of retrospective and prospective study

The pharmacokinetic study with Losferron® was performed during the same period as the prospective study. However, the iron status of the patients included in this study was not improved to the same extent as in the patients from the prospective study. This can be explained by the fact that only obese individuals who already suffered from iron deficiency (ferritin < 30 µg/L and/or transferrin saturation < 20%) were included in this study. Therefore, we can not generalize the results for the iron status from this pharmacokinetic study with Losferron® to all RYGB-patients. In the PK-study, we have shown that the oral exposure of iron gluconate from an effervescent tablet was not altered after RYGB compared to baseline. This could be explained by the elimination of the need for drug disintegration and dissolution which may subsequently have increased absorption. This is comparable with a previous study with calcium citrate performed in RYGB patients, in which the bioavailability for calcium citrate from an effervescent formulation was superior to the bioavailability from a tablet formulation [136].

COMPREHENSIVE ASSESSMENT OF IRON STATUS IS REQUIRED TO DIAGNOSE IRON DEFICIENCY

Furthermore, we have learned that an overall approach to diagnose iron deficiency is very important. In the retrospective study, the determination of iron deficiency was only based on serum ferritin concentrations. However, in obese patients, the concentration of serum ferritin as an acute phase reactant might be increased despite an iron deficiency, and this is also the fact for hepcidin [128;161]. This can be explained by the chronic low-grade inflammation that is associated with obesity, resulting in increased ferritin and hepcidin concentrations. Therefore, it is important to include more parameters than only ferritin to determine iron deficiency. Including C-reactive protein (CRP) can already help to indicate the presence of an underlying inflammation. In the prospective study and

the pharmacokinetic study with Losferron®, we also included transferrin saturation as an iron status marker.

RYGB AFFECTS THE DISPOSITION OF BASIC DRUGS WHICH ARE HIGHLY DEPENDENT ON THE ACIDIC ENVIRONMENT IN THE STOMACH

In section 1.5, we already showed that a gastric bypass influences a lot of pharmacokinetic parameters, which may result in changes in oral drug bioavailability. As the knowledge regarding the impact of gastric bypass on drug disposition is limited and disparate, the current PhD project aimed to investigate systematically the disposition of different model compounds. The choice of the model compounds was based on the Biopharmaceutical Classification System (BCS), as shown in Figure 20.

Figure 20: Overview of model compounds studied in the PK-study, based on the Biopharmaceutical Classification System

No significant differences in oral drug disposition before and after RYGB were observed for the BCS class I compound, metoprolol, neither with the immediate nor with the controlled release formulation. The mean pharmacokinetic parameters of fenofibrate, a BCS class II compound highly dependent on bile salt concentrations for its solubility, were unaltered after RYGB as well. However, the oral exposure of posaconazole was decreased after RYGB. Posaconazole is dependent on intraluminal pH and gastric residence time for its solubility and absorption [188]. Both factors are influenced after RYGB as discussed previously in Chapter 1.5.1. Gastric acid secretion in RYGB

patients is negligible resulting in an elevated gastric pH. This is further enhanced by the widespread use of antacid medication following surgery. The increased gastric pH has an impact on the solubility of drugs, including posaconazole [188;190]. Moreover the gastric residence time is reduced after RYGB; previous studies have shown that the gastric emptying for fluids is decreased post-RYGB [18;62-65]. Caution is therefore needed when prescribing basic drugs which are highly dependent on the acidic environment in the stomach for dissolution/solubility. The disposition of these drugs might be decreased, resulting in under-dosing. To estimate to what extent to oral exposure might be influenced after RYGB, physiologically-based pharmacokinetic (PBPK) modelling and simulation approach can be very useful. In our project, the *in vivo* data of metoprolol (immediate and controlled release) were compared with predictions from the PBPK modeling and the results were similar. Thus, PBPK modeling could be used for dose adjustments following RYGB to avoid potential dangerous over- or underdosing.

11.2 Methodological considerations

In this section, the overall strengths and limitations of the project will be discussed.

QUANTITATIVE AND QUALITATIVE RESEARCH

This project provides a comprehensive overview of different aspects regarding the influence of RYGB by using a combination of qualitative and quantitative research. The advantage of the qualitative part was that in-depth knowledge regarding current practice and experiences was obtained. The results of this part underpinned the necessity of the current PhD project as limited knowledge about nutritional support and adapting drug regimens was often mentioned by the interviewed HCPs as a bottleneck in care. The quantitative part of this project provides measurements of the effects of RYGB on different variables regarding dietary intake, drug and supplement use and pharmacokinetics.

COMPREHENSIVE OVERVIEW OF INTAKE OF MICRO- AND MACRONUTRIENTS BEFORE AND AFTER RYGB

Regarding the intake of micro- and macronutrients, we have collected extensive, reliable data as we calculated dietary and supplement intake on different time points of 54 patients. As these calculations are based on the input of participants, one could argue that incorrect reporting of the effective food intake could have resulted in over- and/or underestimating macro- and micronutrient intake. Nonetheless, no other methods than participant's reporting are currently available to measure dietary intake, except from the use of Doubly-Labelled Water for energy and nitrogen determination in urine for protein. To increase the adherence for the completion of the dietary records, we have chosen to use 2-days dietary records to have a realistic and reliable estimation, because when patients need to fill in a dietary record during a longer period, the adherence to the recording reduces [139].

Another important strength of our approach is that we have estimated the usual intake, based on the collected individual intake. So, our analyses are based on a population distribution. This is an added value compared to previous studies, in which the actual intake was used for the analyses. The use of usual intake is recommended by the Institute of Medicine (IOM) to determine the prevalence of inadequate nutrient intake. Furthermore, we included supplement and drug use to have a complete overview of micronutrient intake. Both, the transformation to usual intake and the inclusion of drug and supplement intake, allow our data to be used for the development of food-based dietary guidelines for bariatric patients in different phases.

Furthermore, we have also collected data about status markers, including hepcidin. To our knowledge, it is the first time that hepcidin has been determined in RYGB-patients. Many previous studies focused either on dietary intake or on general status markers, while we have analyzed both.

In this study, patients were followed at different time points until 1 year post-RYGB. A longer follow-up could be useful to analyze to what extent food content changes contribute to weight regain.

Furthermore, it would give information about the evolution of micronutrient intake after one year post-RYGB.

We collected data about dietary intake and body composition and analyzed correlations between both. Nonetheless, it would be interesting to collect data on physical activity as well. This would give a more complete overview of factors influencing body composition, because physical activity has also an important influence on body composition.

SYSTEMATIC APPROACH FOR THE PHARMACOKINETIC STUDY

To our knowledge, the pharmacokinetic study of this project is the first pharmacokinetics study in bariatric patients with a systematic approach for different drugs. First, we used the same design for all drugs. Second, we followed the same patient group before and after surgery to exclude inter-individual differences. Moreover, in the included patients, the same type of surgery, namely a laparoscopic RYGB with an alimentary limb of 120 cm and a small gastric pouch, was performed by the same surgeon according the same procedure. Furthermore, the postoperative time points were comparable in all included patients. These similarities are an important strength of our methodology as it makes it possible to compare results from the different drugs. The choice of the different drugs was based on the Biopharmaceutical Classification System (BCS), which classifies drugs according to their solubility and permeability. The model compounds were therefore chosen based on their absorption characteristics, rather than on their use in clinical practice.

In this project, we have performed a single-dose pharmacokinetic study before RYGB and at one time point after surgery. Repeating the single-dose pharmacokinetic study at an additional time point postoperatively could be interesting as in previous pharmacokinetic studies in RYGB-patients, different results were observed at different time points postoperatively, such as for SSRI [96]. A possible explanation for these differences may be found in the development of adaptation mechanisms for absorption.

COMBINATION OF *IN VIVO* DATA WITH PREDICTED DATA

We combined *in vivo* data from the performed pharmacokinetic study with metoprolol with predicted results from physiologically-based pharmacokinetic (PBPK) modelling and simulation. The PBPK modelling and simulation is ongoing for fenofibrate and posaconazole; combination of the predicted results with the *in vivo* data for these compounds will soon be possible. This combination serves as an ongoing validation of a previously developed PBPK model [71] for predicting oral drug exposure following RYGB as *in vivo* data are scarce. Physiologically-based pharmacokinetic modelling and simulation could be used for dose adjustments following RYGB to avoid potential dangerous over- or under-dosing. Thus, it might be very useful in clinical practice.

11.3 Recommendations

CARE PATHWAY

In the qualitative part of this project, we found that there is a wide variation regarding screening and follow-up of patients before and after bariatric surgery in Flanders. Nowadays, only approval from the surgeon, internist, and psychologist/psychiatrist are required for reimbursement of the surgery and no follow-up consultations are obliged, as discussed in Chapter 10. Therefore, we recommend an adequate and more universal multidisciplinary approach before and after bariatric surgery, which can be enabled by the development of a care pathway. Our proposal for a possible overall care pathway is shown in Figure 21. First, it is essential to check whether the patient meets the eligibility criteria. If the patient meets the criteria, information about beneficial and potentially adverse effects of bariatric surgery need to be explained and one must be sure that the patient understands the impact of the procedure. Further, the patient needs to be screened and evaluated by a multidisciplinary team, at least consisting of a surgeon, endocrinologist, psychologist and dietitian. Including a physiotherapist and a clinical pharmacist in this team could be of additional value.

A multidisciplinary meeting should be organized on a regular basis (i.e. weekly for large bariatric centers and once or twice a month for smaller bariatric centers) with all involved HCPs to discuss all patients who have presented with a question to undergo bariatric surgery. If the patients have been evaluated by all involved HCPs, a thorough concertation can take place, resulting in a personalized proposal for each patient, i.e. bariatric surgery or an alternative treatment. If bariatric surgery is recommended, the type of surgery needs to be chosen in function of the characteristics of the patient.

After bariatric surgery, a proper follow-up by a multidisciplinary team is recommended to maximize outcomes and minimize complications, especially on the long-term. It is important that the tasks for each HCP are clearly defined to provide an extensive screening and a proper, universal follow-up.

Furthermore, it is important to involve HCPs from primary care in the whole care pathway.

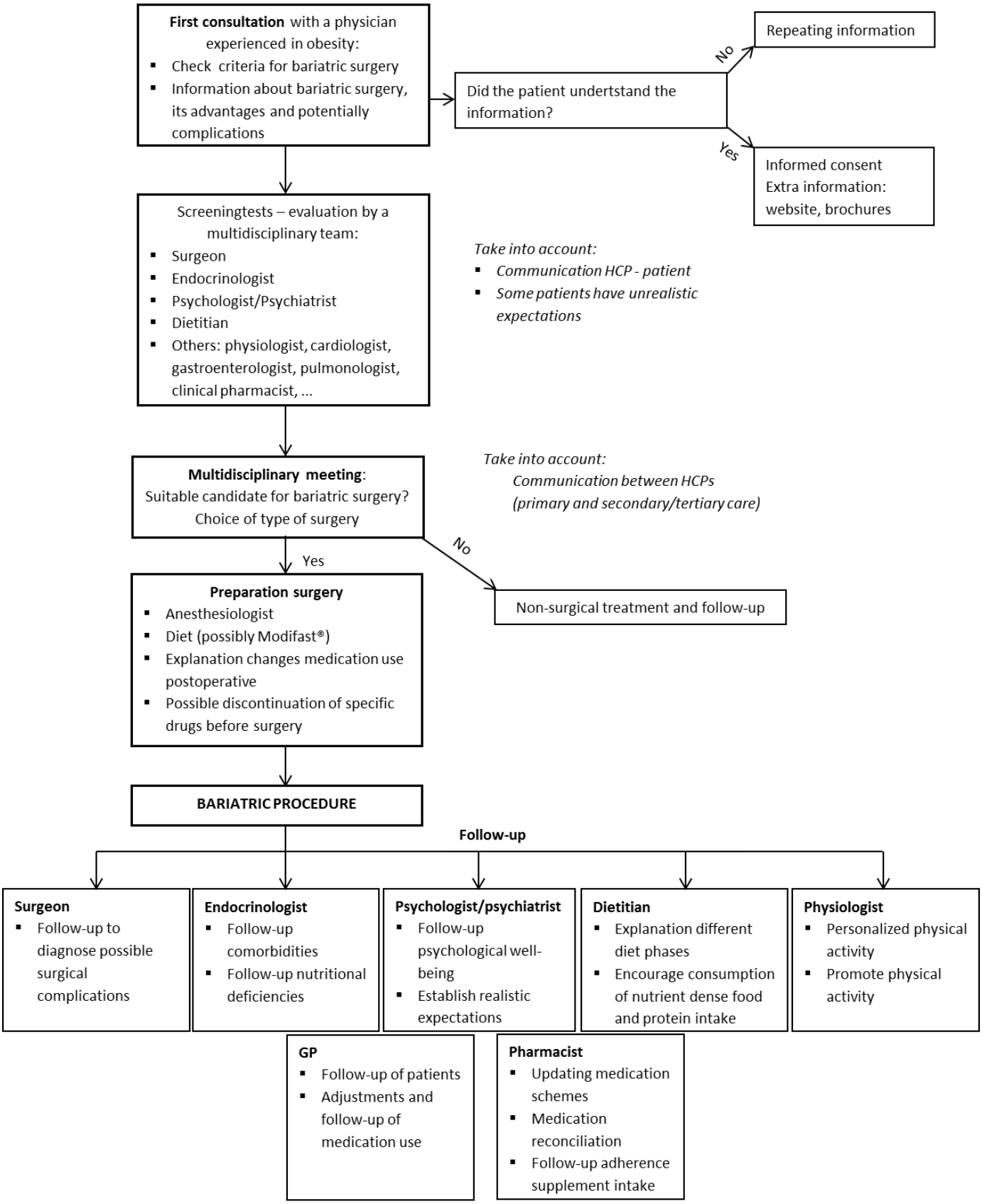


Figure 21: A possible care pathway for patients before and after bariatric surgery

BALANCE BETWEEN SECONDARY/TERTIARY AND PRIMARY CARE

Because of the growing number of patients with a bariatric surgery history, it is no longer possible to organize the entire follow-up of all these patients in secondary/tertiary care. Therefore, an elaboration of the collaboration between secondary/tertiary care and primary care (involving the GP, pharmacist, dietitian and psychologist) is needed. To optimize this collaboration, HCPs from primary care need to be involved already from the beginning. A proposal of a possible balance between input from primary and secondary/tertiary care, is shown in Figure 22.

A central idea in this model is that the longer the time after surgery, the more important the role of primary care will be. However, HCPs of primary care need to have sufficient knowledge about complications and follow-up of bariatric patients as for example these patients can suffer from nutritional deficiencies that are not common in the general population, such as vitamin B_1. To increase this knowledge, educational sessions and skills enhancement activities should be given to HCPs of primary care, and care goals should be clearly shared.

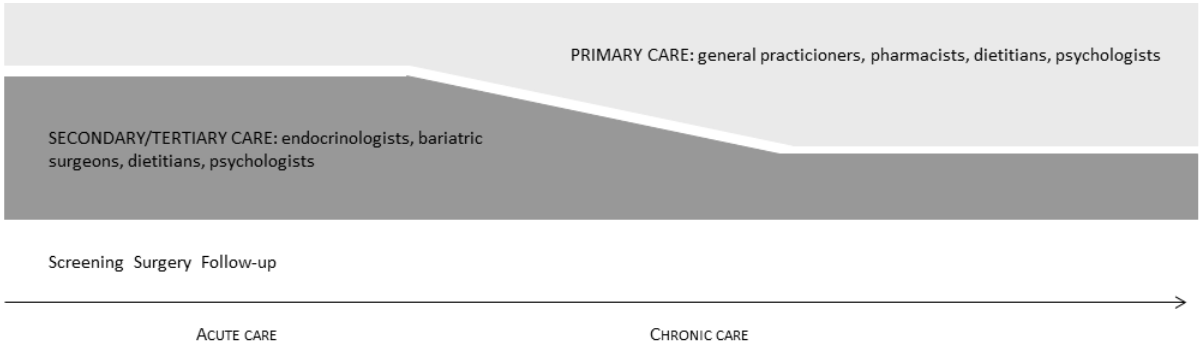


Figure 22: Collaboration primary and secondary/tertiary care

It is also important that all HCPs are aware that a patient has undergone bariatric surgery in order to work in accordance with the needs after bariatric surgery. To communicate to other HCPs that a bariatric procedure has been performed, a medical passport, indicating which procedure has been performed and what the main attention points are, could be helpful. This passport could be carried by the patient. An example of such a medical passport for patients with RYGB is shown in Figure 23.

In the future, this type of information should be available in a patients' electronic health record, to be shared between all HCPs involved.

MEDICAL PASSPORT

Mrs. X (28 years)

Roux-en-Y gastric bypass (RYGB)

⇒ Formation of small gastric pouch

⇒ Bypass of duodenum and proximal jejunum

Performed on 01/01/2015 in the University Hospitals Leuven by Dr. Matthias Lannoo

Contact person (+tel): Mrs. X – 0488/12 34 56

ADVICE

- Encourage consumption of **nutrient dense food**
- Stimulate **protein intake** to preserve lean body mass
- Extensive **biochemical follow-up** to diagnose nutritional deficiencies as early as possible
- Life-long **multivitamin supplement** and calcium (**calcium citrate** is preferred)
- **Avoid NSAIDs** → prefer paracetamol (severe pain: tramadol)
- Attention for the **size of drugs** (especially the first months after RYGB) → prefer orodispersible tablets, effervescent tablets (completely effervesced with minimal carbonation) syrups, suppositories, crushing tablets if allowed
- **Adequate contraception** to avoid pregnancy the first 18 months post-RYGB and unwanted pregnancies (oral contraception might be less effective post-RYGB)

Figure 23: Medical passport

EXTENSION OF FUNDING

The qualitative interviews performed in this project have shown a large variation regarding multidisciplinary approach in patients before and after bariatric surgery. In almost all participating hospitals, a multidisciplinary team was involved in the care for bariatric surgery patients. However, there was a large variation in multidisciplinary approach i.e. differences in the composition of the multidisciplinary team and in the organization of multidisciplinary meetings. A multidisciplinary meeting has several advantages, but some bottlenecks mentioned by the interviewed HCPs, need to be addressed. One of the most important ones is probably funding. Reimbursement of multidisciplinary meetings could facilitate collaboration, and result in a more universal multidisciplinary approach of patients before and after bariatric surgery. Furthermore, reimbursement of follow-up consultations, especially for dietitian and psychologist, could optimize long-term follow-up.

NUTRITIONAL GUIDANCE

Our study showed that dietary intake of macronutrients and micronutrients (iron, vitamin B_{12}, vitamin C, copper and zinc) was markedly decreased after RYGB, indicating that nutritional guidance is essential following bariatric surgery. Because of the reduced food intake after bariatric surgery, we believe that it is important to optimize the diet of these patients by encouraging the intake of nutrient dense food and stimulating adjustments in eating behavior. Although fruit and vegetable intake are part of the recommended diet, the fiber intake (such as in fruits, vegetables and whole grains) needs to be increased and deserves specific attention as almost all patients had an inadequate fiber intake. A dietitian can play a major role in this regard, by educating and counseling patients to adjust their eating behavior, as to optimize outcomes and minimize complications of surgery [244-246]. Furthermore, nutritional follow-up on the long-term is important as it is essential to prevent weight regain. Our results indeed indicated a recurrence of eating habits with time after surgery as the distribution of the energy percentage from the different macronutrients gradually tempted to the distribution from before surgery.

Secondly, we showed that some patients had an inadequate intake of micronutrients, compared to the age- and gender- specific EAR [105], despite the recommendation to use supplements. In these patients, stimulating adherence of supplement use is necessary to obtain the requirements for micronutrient intake and subsequently to prevent the development of micronutrient deficiencies [36]. Furthermore, we have shown a significant correlation between protein intake and FFMI. Therefore, supporting protein intake is very important in this population group in order to preserve lean body mass. Likewise, limiting fat intake in this population group should prevent weight regain. Overall, it is important that all the nutrition advice is given to patients in a convenient way, and that no burden is created in order to keep patients motivated.

FOLLOW-UP OF MEDICATION USE

The performed qualitative interviews have shown that medication use is questioned in clinical practice at screening consultations in Belgium. However, in most of the hospitals, no pharmacist is involved in the screening and follow-up of patients to obtain an accurate overview of the medication use. Nevertheless, Silverman et al. have shown that collaboration between surgeons and pharmacists improved pharmaceutical care in these patients as the pharmacist suggested to crush medication, to change the formulation and gave other relevant pharmaceutical advice [250]. In this project, we have also demonstrated that medication use changes a lot after bariatric surgery since the improvement of obesity related diseases and the increased use of multivitamin/mineral supplements. Therefore, a good follow-up regarding medication use is also essential, preferentially by a (clinical) pharmacist. We believe that it would be helpful to make medication schemes for this population group to prevent errors in medication use, and to share these schemes with other HCPs in order to avoid conflicting information. Sufficient information needs to be provided in these schemes, not only listing the current medication and supplement use, but also adjustments that have been made and targets that need to be reached i.e. for blood pressure. As the medication use changes a lot after bariatric surgery, it is also important to keep the medication schemes up to date.

Because of all the physiologic changes related to bariatric surgery, it is very important for clinicians to monitor patients with a RYGB for drug efficacy and toxicity and when required, to adjust their medication. If the oral administration of a drug is not associated with the desired effect, it could be caused by a reduction in drug disposition. Subsequently, a switch to another formulation or another administration route should be considered.

Based on our pharmacokinetic studies, HCPs need to be careful for under-dosing in post-RYGB patients when the solubility of medication is dependent on intraluminal pH and/or gastric residence time. If the solubility of the drug is only dependent on bile acids, no significant decrease in the mean oral exposure was observed in this study. However, further research is necessary to investigate the influence of RYGB on the secretion of bile acids in the small intestine. Administration of a drug in a

controlled release formulation based on a matrix system did not result in no reduced oral exposure as the compound on itself has no decreased oral exposure after RYGB. However, the formulation of drugs and supplements needs to be considered as it can influence the extent of absorption. An effervescent tablet seemed to give less absorption problems after RYGB compared to a tablet formulation, as shown in the pharmacokinetic study with Losferron®. However, further research is necessary as many questions remain, as discussed in section 1.4.

11.4 Future perspectives

SCIENTIFIC RESEARCH

Need for data on effectiveness of drugs

In this project, we have focused on pharmacokinetics, but in the future, it would be interesting to combine data about pharmacokinetics and pharmacodynamics, more specifically about the effectiveness of drugs for which the disposition is (potentially) impaired after RYGB. One of the drug classes for which knowledge about the effectiveness after bariatric surgery is highly needed, is (oral) hormonal contraception. Therefore, we have planned a study in which we will investigate the pharmacokinetics of combined oral contraception together with measurements of the inhibitory effect on the ovulation in women before and after bariatric surgery.

Need for data on the impact of sleeve gastrectomy

While information regarding pharmacokinetics and nutrient absorption post-RYGB is scarce, this information is almost nonexistent for sleeve gastrectomy. According to our knowledge, there is only one case report regarding pharmacokinetics after sleeve gastrectomy, showing altered pharmacokinetics of imatibin mesylate postoperatively [252]. Hence, a comparison of pharmacokinetics after RYGB and sleeve gastrectomy would be interesting. From a theoretical point of view, sleeve gastrectomy is thought to have less impact on drug and nutrient disposition as it only affects the stomach by the formation of a gastric tube [12]. Therefore, clinicians often choose to

perform a sleeve gastrectomy instead of a RYGB in patients taking critical medicines. However, there is no evidence to support this hypothesis.

Need for data on biological and pathophysiological parameters after RYGB

Since little is known about drug behavior in patients with deviating GI physiology, this condition is often not taken into account when establishing dosing schemes, resulting in drug underperformance in practice. To fill this gap, it would be interesting to investigate biological and pathophysiological parameters such as pH, metabolites, systemic and gastrointestinal drug concentration over time in bariatric patients. For example, it is known that the serum concentration of bile acids is twice as high after RYGB compared to before surgery [66;67]. However, no information about the concentration of bile acids in the intestine is known. The latter is necessary to have a better understanding of possible adaptation mechanisms and influence on drug solubility and absorption. Likewise, from a theoretical point of view, the gastric mixing will be reduced after RYGB as a part of the stomach is bypassed. It would be useful to study the extent to which this (potentially) reduced gastric mixing has an influence on the disintegration of tablets. This could be done by administration of tablets with different sizes, followed by measurement of gastric mixing by high-resolution manometry (HRM) [163] and determination of drug plasma concentrations.

OPTIMIZING CLINICAL PRACTICE

One of the major challenges in sciences is the translation of knowledge obtained from scientific research to the clinic. An adequate translation should lead to direct implementation resulting in an immediate impact on the patient's health.

Development of a web application with information on (altered) pharmacokinetics

We do believe that the results from pharmacokinetic studies need to be implemented in clinical practice as soon as possible, in order to improve current dosing regimens of selected drugs. This can be done by extrapolating data from pharmacokinetic studies performed in RYGB patients to frequently used drugs in this population group [238]. Combining pharmacokinetic data from a healthy

population and data from previously performed pharmacokinetic studies in RYGB patients of drugs with comparable absorption characteristics should allow to predict if drug disposition is altered after RYGB. Data from PBPK modelling can also be used to create this overview. Generating this evidence for drugs that are frequently used in this population group, should result in an easy accessible database, which allows clinicians to look up pharmacokinetic information of selected drugs, and to support decisions on dose adjustments in order to avoid potentially dangerous over- or under-dosing. To ensure that this information is available for all HCPs, it should be accessible from a web application. In Table 34, an example for the construction of such an overview is displayed.

Table 34: Example for the construction of an overview of the influence of RYGB on the disposition of drugs

Compound	BCS	Level of evidence	Effect on pharmacokinetics	Implications for clinical practice
Rivaroxaban	Class II	Case-report [74]	3 months post-RYGB: after first administration, C_{max} was 224 ng/mL and after the second administration 262 ng/mL, with an immediate effect on INR	No dose adjustments seem to be necessary after RYGB
Itraconazole	Class II	PK-study with posaconazole, a compound with comparable absorption characteristics [253]	6 months post-RYGB: the oral disposition of posaconazole was significantly decreased	Monitoring could be useful to prevent underdosing of itraconazole

Clinical studies in obese individuals before registration of medicinal products

There is a trend from the FDA and EMA that studies need to be performed in specific groups of patients before registration of medicinal products, such as the EU Paediatric Drug Regulation on the marketing authorization of drugs for children [254]. Since there is hardly any information available in the growing population of obese individuals, it would be interesting that additional studies in this patient group are also required for marketing authorization of drugs. In this way, obese individuals can also receive appropriate drug information and the knowledge regarding influence of obesity on pharmacokinetics would be extended.

Development of food-based dietary guidelines and dietary recommendations for RYGB patients

Our data regarding nutrient intake from food and supplements in combination with the status data can and should be used for the development of food-based dietary guidelines and dietary recommendations for this specific population group. According to the conceptual framework of the EURRECA Network of Excellence dietary guidelines could be derived from our data [140]. Food-based dietary guidelines are intended to establish a basis for nutrition education programs to foster healthy eating habits and lifestyles. These guidelines would be of added value to the bariatric food pyramid, developed by. Moizé et al., which is a 'one size fits all' model and is not suitable for the changing dietary advices in the first year after surgery [255].

SUMMARY

The worldwide epidemic of obesity is associated with an increased demand for bariatric surgery. Nowadays, a Roux-en-Y gastric bypass (RYGB) is the most performed bariatric procedure. It is associated with anatomical changes of the gastrointestinal tract by the formation of a small gastric pouch and a bypass of the proximal part of the small intestine. These changes will influence the intake and absorption of macro- and micronutrients and drugs. Therefore, the overall of this project was to understand the effect of Roux-en-Y gastric bypass on food intake, medication/supplement use and oral drug disposition.

The project consisted of 6 major parts. In **part I**, an overview of the current knowledge regarding the influence of RYGB on the intake and absorption of macro- and micronutrients and on its influence on medication use and pharmacokinetics, is shown.

The results described in **part II** derive from the prospective study, in which data regarding dietary intake, medication and supplement use, body composition and status markers were collected, both before and at different time points post-RYGB. The energy and macronutrient intake was significantly decreased one month post-RYGB and gradually increased again with time after surgery. The fat mass index (FMI) significantly decreased until one year post-RYGB and the fat free mass index (FFMI) significantly decreased until 6 months post-RYGB. A significant correlation was observed between protein intake and FFMI; emphasizing the importance of stimulating RYGB-patients for an adequate protein intake.

Furthermore, the dietary intake of iron, vitamin B_{12}, vitamin C, copper and zinc was markedly decreased after RYGB. By including supplement intake of the micronutrients, there were still some patients with an inadequate intake of iron, copper and vitamin C one year post-RYGB, compared to the age- and gender- specific Estimated Average Requirements (EAR). Our data clearly suggest that medical nutrition therapy is essential following bariatric surgery. Moreover, the iron status was improved after RYGB, characterized by an increase in transferrin saturation and a decrease in serum hepcidin concentration. The latter reflects the improvement of the chronic obesity-associated

inflammation and is a potential indication for enhanced iron absorption after RYGB and subsequently improved iron profile.

Iron deficiency is a frequent complication after RYGB, necessitating a good follow-up of these patients in order to diagnose and to treat these complications. In **part III**, we showed that female gender, young age, preoperative poor iron status, vitamin B_{12} deficiency, and the time post-RYGB are predisposing factors for the development of iron deficiency. Iron supplementation seems essential in this population, but the effect of oral tablets may be limited. In the oral iron absorption tests, we have shown that the absorption of iron from a tablet formulation was low post-RYGB. We have also performed a pharmacokinetic study to evaluate the disposition of iron gluconate from an effervescent tablet before and after RYGB. No significant differences in iron disposition were observed in this study. This could be explained by the elimination of the need for drug disintegration and dissolution as a result of the liquid formulation. The formulation of supplements and drugs need to be considered in patients with RYGB.

We have also performed pharmacokinetic studies with model compounds, which are reported in **part IV.** The choice of model compounds was based on the Biopharmaceutical Classification System (BCS), which classified drugs according to their solubility and permeability.

For metoprolol, a BCS class I compound, which is characterized by a high solubility and high permeability, the oral exposure was not significantly different before compared to after RYGB, neither the immediate, nor the controlled release formulation. Although a tendency towards higher exposure existed following surgery, which could be explained by weight loss and a reduced presystemic biotransformation in the proximal GI-tract. The *in vivo* data were compared with predicted results from the physiologically-based pharmacokinetic modelling and simulation for the immediate and controlled metoprolol formulations. The predicted values were similar to the observed data.

Pharmacokinetic studies were also performed with two compounds belonging to BCS class II, fenofibrate and posaconazole. Both compounds are characterized by a low solubility and high permeability; with the difference that fenofibrate is a neutral compound and posaconazole a weak base. The pharmacokinetic parameters for the disposition of fenofibrate, highly dependent on bile salt concentrations for its solubility, were not significantly different after RYGB. This is in contrast with the significantly decreased oral exposure of posaconazole after RYGB, which could be explained by the increased gastric pH and accelerated gastric emptying of fluids postoperative as the systemic exposure of posaconazole is related to the residence time in the acidic environment of the stomach.

The aim of **part V** was to get insight in the influence of RYGB on medication use and the associated costs. Medication and medical devices prescribed for obesity-related diseases and for the prevention of surgical and nutritional complications was taken into account. We showed that RYGB induces a significant reduction of total medication costs, as early as 1 year post RYGB, compared to baseline. Importantly, the reduced medication cost was maintained over several years post-RYGB and appears mainly to be due to the significant improvement of diabetes type 2 and obstructive sleep apnea. Hence, RYGB entails economic benefits by reducing the costs and need for medication and medical devices.

The have an overview of the current clinical practice of bariatric surgery patients concerning screening and follow-up, qualitative interviews were performed, described in **part VI.** The current care for bariatric patients in Flemish hospitals, both before and after surgery, varies widely including nutritional guidance, composition of multidisciplinary team and the presence of multidisciplinary meetings. Strategies to implement existing guidelines in clinical practice are required in order to obtain more uniform, multidisciplinary support for bariatric patients, resulting in an increase of efficiency and improved patient care.

Recommendations for the clinical practice are proposed in the closing **part VI**. A more universal multidisciplinary approach is recommended in patients before and after bariatric surgery. This can be

realized by the development of a care pathway. An extension of funding can help to develop a multidisciplinary care pathway and to improve follow-up of bariatric patients. Moreover, an elaboration of the collaboration between secondary/tertiary care and primary care is needed to facilitate long-term follow-up of these patients, including nutritional guidance to optimize their outcomes and minimize complications of bariatric surgery, and a good follow-up regarding medication use.

Upcoming challenges for scientific research and optimizing clinical practice are also discussed in part VI. Pharmacokinetic studies need to be combined with pharmacodynamic studies in this population, more specifically about the effectiveness of drugs for which disposition is (potentially) impaired after RYGB. Furthermore, pharmacokinetic studies in patients with sleeve gastrectomy need to be performed, since nowadays this information is almost non-existent. Future research could focus on investigation of biological and pathophysiological parameters such as pH, metabolites, systemic and gastrointestinal drug concentrations over time in bariatric patients.

To optimize clinical practice, a web application available for all HCPs with information on (altered) pharmacokinetics can be developed. Patients with bariatric surgery need to be encouraged to adopt healthy eating habits and lifestyles. Therefore, the development of food-based dietary guidelines and dietary recommendations for this specific population group is essential. Moreover, to gain knowledge regarding pharmacokinetics in obese individuals, it would be interesting that additional studies in this patient group are also required for marketing authorization of drugs.

SAMENVATTING

De wereldwijde epidemie van obesitas is geassocieerd met een verhoogde vraag naar bariatrische chirurgie. Vandaag de dag is een Roux-en-Y gastric bypass (RYGB) de meest uitgevoerde bariatrische ingreep. Deze ingreep gaat gepaard met anatomische veranderingen van het gastro-intestinale stelsel door de vorming van een klein maagzakje en een bypass van het proximale deel van de dunne darm. Deze veranderingen zullen de inname en absorptie van macro- en micronutriënten en geneesmiddelen beïnvloeden.

Het doel van dit project was om het effect van een RYGB na te gaan op voedselinname, geneesmiddelen- en supplementengebruik en op de orale dispositie van geneesmiddelen.

Het project bestaat uit 7 grote delen. In **deel I** wordt een overzicht gegeven van de huidige kennis over de invloed van RYGB op de inname en absorptie van macro- en micronutriënten en over de invloed van de ingreep op het geneesmiddelengebruik en de farmacokinetiek.

In **deel II** worden de resultaten beschreven die verkregen zijn uit de prospectieve studie, waarin gegevens betreffende voedingsinname, geneesmiddelen- en supplementengebruik, lichaamssamenstelling en status markers werden verzameld, zowel voor als op verschillende tijdstippen na RYGB. De energie- en macronutriënteninname waren significant afgenomen één maand na RYGB en nam geleidelijk aan weer toe met de tijd na de ingreep. De vetmassa-index daalde significant tot en met één jaar na de RYGB en de vetvrije massa-index daalde significant tot en met 6 maanden na de ingreep. Een significante correlatie tussen de eiwitinname en de vetvrije massa-index werd waargenomen, wat het belang van het stimuleren van een adequate eiwitinname bij patiënten met een RYGB benadrukt.

Bovendien is de inname via de voeding van ijzer, vitamine B_{12}, vitamine C, koper en zink duidelijk verminderd na RYGB. Zelfs wanneer de micronutriënteninname via supplementen mee in rekening werd gebracht, waren er, op basis van de vergelijking met de leeftijd- en geslachts-specifieke 'Estimated Average Requirements' (EAR), nog steeds sommige patiënten met een inadequate inname van ijzer, koper en vitamine C één jaar na RYGB. Onze gegevens suggereren duidelijk dat medische

voedingstherapie essentieel is na bariatrische chirurgie. Daarnaast stelden we vast dat de ijzerstatus verbeterd was in de patiënten na RYGB, wat gekenmerkt werd door een toename in transferrine saturatie en een vermindering in de serumconcentratie van hepcidine. Deze verminderde hepcidine concentratie reflecteert de verbetering van de chronische inflammatie die aan obesitas gerelateerd is, en is een potentiële indicatie voor een betere ijzerabsorptie na RYGB, wat mogelijk resulteert in een verbeterde ijzerstatus.

Ijzerdeficiëntie is een veel voorkomende complicatie na RYGB, waardoor een goede follow-up van deze patiënten noodzakelijk is, zodat tijdige diagnose en behandeling mogelijk zijn. In **deel III**, hebben we aangetoond dat het vrouwelijke geslacht, jonge leeftijd, een slechte ijzerstatus preoperatief, een vitamine B_{12}-tekort, en de tijd na RYGB predisponerende factoren zijn voor de ontwikkeling van een ijzertekort postoperatief. Het gebruik van ijzersupplementen lijkt essentieel in deze populatie, maar het effect van orale tabletten is mogelijk beperkt. In de orale ijzerabsorptietesten, hebben we aangetoond dat de absorptie van ijzer uit een tablet post-RYGB beperkt was. In een farmacokinetische studie met ijzergluconaat onder de vorm van een bruistablet vonden we echter geen significante verschillen in de dispositie van ijzer. Dit kan wellicht verklaard worden door het feit dat de noodzaak voor desintegratie en het in oplossing gaan van ijzer vermeden wordt, aangezien het geneesmiddel wordt toegediend in een vloeibare vorm. Daarom moet er goed worden nagedacht over de keuze van formulering van supplementen en geneesmiddelen bij patiënten na een RYGB.

In **deel IV** rapporteren we de resultaten van farmacokinetische studies met verschillende modelcomponenten**.** De keuze van deze modelcomponenten was gebaseerd op het Biofarmaceutische Classificatiesysteem (BCS), dat geneesmiddelen classificeert op basis van oplosbaarheid en permeabiliteit.

Voor metoprolol, behorend tot BCS klasse I en gekenmerkt door een hoge oplosbaarheid en een hoge permeabiliteit, werd er geen significant verschil waargenomen in orale blootstelling voor en na

RYGB, niet vanuit de formulering met directe afgifte en ook niet vanuit de formulering met een gecontroleerde afgifte. Er was echter wel een tendens voor een verhoogde blootstelling na de ingreep, die kan verklaard worden door gewichtsverlies en een verminderde presystemische biotransformatie in het proximale deel van het gastro-intestinale stelsel. Deze *in vivo* gegevens werden verder vergeleken met voorspelde resultaten, die verkregen werden op basis van een op fysiologie gebaseerde farmacokinetische modellering en simulatie. De voorspelde waarden waren vergelijkbaar met de waargenomen gegevens.

We hebben ook farmacokinetische studies uitgevoerd met twee modelcomponenten behorend tot BCS klasse II, namelijk fenofibraat en posaconazole. Beide geneesmiddelen worden gekenmerkt door een lage oplosbaarheid en een hoge permeabiliteit; met het verschil dat fenofibraat een neutrale verbinding is en posaconazole een zwakke base. Er werden geen significante verschillen in de farmacokinetische parameters voor de dispositie van fenofibraat waargenomen na RYGB, alhoewel fenofibraat voor zijn oplosbaarheid sterk afhankelijk is van de concentratie aan galzouten. Dit is in tegenstelling tot de sterk verminderde orale blootstelling van posaconazole na RYGB, die kan verklaard worden door de verhoogde pH in de maag en de versnelde maaglediging voor vloeistoffen postoperatief. De systemische blootstelling aan posaconazole is immers gerelateerd aan de verblijftijd in de zure omgeving van de maag.

Het doel van **deel V** was om de invloed van een RYGB op het geneesmiddelengebruik en de daarmee gepaarde kosten te analyseren. Zowel het gebruik van geneesmiddelen en medische hulpmiddelen voorgeschreven voor obesitas gerelateerde aandoeningen als voor de preventie van chirurgische en nutritionele complicaties werden in kaart gebracht. We hebben aangetoond dat een RYGB leidt tot een significante vermindering van de totale geneesmiddelenkosten vanaf 1 jaar postoperatief, vergeleken met de geneesmiddelenkost preoperatief. Deze geneesmiddelenkosten bleven significant verlaagd gedurende meerdere jaren na RYGB en dit was voornamelijk te wijten aan de significante

verbetering van diabetes type 2 en slaapapneu. Een RYGB brengt dus economische voordelen met zich mee door het verminderen van de kosten voor geneesmiddelen en medische hulpmiddelen.

Om een overzicht van de huidige klinische praktijk met betrekking tot de screening en opvolging van bariatrische patiënten te verkrijgen, werden kwalitatieve interviews uitgevoerd, beschreven in **deel VI**. De huidige zorg voor bariatrische patiënten in Vlaamse ziekenhuizen varieert sterk en dit zowel voor als na de operatie. De verschillen worden gezien in nutritionele begeleiding, de samenstelling van het multidisciplinaire team en de organisatie van multidisciplinaire meetings. Het is dan ook noodzakelijk om strategieën te ontwikkelen om bestaande richtlijnen te implementeren in de klinische praktijk om een meer uniforme, multidisciplinaire begeleiding voor bariatrische patiënten te kunnen realiseren. Dit zou moeten leiden tot een toename van efficiëntie en verbeterde patiëntenzorg.

Aanbevelingen voor de klinische praktijk worden in het laatste deel voorgesteld, **deel VII**. Centraal staat een meer universele multidisciplinaire aanpak voor patiënten voor en na bariatrische chirurgie wat kan gerealiseerd worden door het ontwikkelen van een zorgpad. Extra financiering zou de ontwikkeling van dergelijk zorgpad kunnen faciliteren en de follow-up van deze patiënten kunnen verbeteren. Bovendien is het uitwerken van de samenwerking tussen tweede/derde lijn en eerste lijn nodig om follow-up op lange termijn, met inbegrip van nutritionele begeleiding, te bestendigen. Dit moet er voor zorgen dat de resultaten van bariatrische chirurgie geoptimaliseerd worden en de complicaties tot een minimum beperkt worden. Een goede follow-up met betrekking tot het geneesmiddelengebruik maakt daar vanzelfsprekend deel van uit.

Toekomstige uitdagingen op vlak van wetenschappelijk onderzoek en optimalisatie van de klinische praktijk worden eveneens besproken in deel VII. Farmacokinetische studies zouden gecombineerd moeten worden met farmacodynamische studies in deze populatie, vooral om de effectiviteit van geneesmiddelen na te gaan waarvoor de dispositie (mogelijks) gewijzigd is na RYGB. Bovendien zouden farmacokinetische studies in patiënten met een sleeve gastrectomie nuttig zijn, aangezien

informatie hierover vrijwel onbestaande is. Het zou eveneens interessant zijn om toekomstig onderzoek te richten op (veranderingen in) biologische en pathofysiologische parameters zoals pH, metabolieten en systemische en gastro-intestinale geneesmiddelenconcentraties in bariatrische patiënten. Om de klinische praktijk te optimaliseren, kan een webapplicatie ontwikkeld worden die beschikbaar is voor alle zorgverleners en informatie over (veranderde) farmacokinetiek in deze patiëntenpopulatie. Verder moeten patiënten met bariatrische chirurgie gestimuleerd worden om gezonde eetgewoonten en een gezonde levensstijl aan te nemen. Daarom is de ontwikkeling van voedingsrichtlijnen en -advies van essentieel belang. Om meer kennis te verwerven over farmacokinetiek in obese individuen, zou het nuttig zijn dat bijkomende studies in deze patiëntengroep vereist worden alvorens geneesmiddelen op de markt komen.

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PROFESSIONAL CAREER

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Education

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LIST OF ORIGINAL PEER-REVIEWED PUBLICATIONS

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Multidisciplinary approach to Obesity: obesity and malabsorption. Advances in Diabetes – Training course. Leuven, 18 February 2014.

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